Blood pressure control during exercise in people with hypertension

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Abstract

An exaggerated blood pressure (BP) response to maximal exercise is an independent risk factor for cardiovascular events and mortality. In people with hypertension, it is unclear if treating BP to guideline levels normalises the rise in BP during exercise, which is mediated in part by the metaboreflex. The main aim of this thesis was to assess whether adequate control of BP with anti-hypertensive medication normalises the exaggerated pressor response to exercise that is well established in untreated hypertension. It was hypothesised that treatments that reduce metaboreflex hyperreflexia would lower the BP response to maximal exercise. The BP response to exercise was assessed during an incremental exercise test to peak oxygen consumption ($\dot{V}O_2$ peak) on a cycle ergometer. To assess the BP response to metaboreflex isolation, post-exercise ischemia following isometric handgrip exercise was used. The first main finding of this thesis was that patients with treated-controlled, uncontrolled and untreated hypertension had an exaggerated BP response to exercise and metaboreflex isolation compared to age matched healthy controls. Secondly, the metaboreflex remains predictive of the peak BP response to incremental exercise when accounting for other known risk factors, including measures of central and peripheral arterial stiffness (aortic pulse pressure & pulse wave velocity), age and daytime ambulatory peripheral systolic BP. Finally, because the metaboreflex hyperreflexia depends on normal blood flow responses to exercise, an aim of this thesis was to improve nitric oxide bioavailability with chronic dietary nitrates. Despite improved levels of plasma nitrates in patients with treated-controlled hypertension, dietary nitrate supplementation had no impact on the maximal BP response to exercise or metaboreflex isolation compared to a placebo. Future research will need to assess alternative therapies to reduce exercise BP in patients with treated-controlled hypertension. This research will hopefully reduce the heightened cardiovascular risk in this population.

Dedication and Acknowledgements

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Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: Ben Chant DATE: 25th October 2018

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Chapter 1 General Introduction

1.1 List of Abbreviations

$[Ca^{2+}]i$	Calcium influx
A	Area
A(P)	Aortic cross-sectional area for pressure
ABPM	Ambulatory blood pressure monitoring
ACE	Angiotensin converting enzyme
AIx	Aortic augmentation index
Akt	Protein kinase B
A_{max}	Maximal diameter of the aorta
ANOVA	Analysis of variance
ANS	Autonomic nervous system
ASIC	Acid sensing ion channel
AT_1 receptor	Angiotensin type 1 receptor
ATP	Adenosine triphosphate
BH_4	Tetrahydrobiopterin
BMI	Body mass index
BP	Blood pressure
C3	Complement factor 3
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate

cGMP	Cyclic guanosine monophosphate
CO_2	Carbon dioxide
CO	Cardiac output
CRiC	Clinical Research and Imaging Centre
CV	Cardiovascular
CVLM	Caudal ventrolateral medulla
CVP	Central venous pressure
C_w	Windkessel (buffer) compliance
DBP	Diastolic blood pressure
ECG	Electrocardiogram
EEG	Electroencephalogram
eNOS	Endothelial nitric oxide synthase
EPC's	Endothelial progenitor cells
GABA	Gamma-Aminobutric acid
Gs	G stimulatory proteins
GTP	Guanosine triphosphate
H	Wall thickness
HF	High-frequency
HR	Heart rate
HRV	Heart rate variability
K	Hydraulic conductance,

L	Length
LF	Low-frequency
L-NAME	N-nitro-L-arginine methyl ester
M_2	Muscarinic 2
MAP	Mean arterial pressure
MAPKs	Mitogen-activated protein kinases
MSNA	Muscle sympathetic nerve activity
MVC	Maximal voluntary contraction
η	Viscosity
NAD(P)H	Nicotinamide-adenine dinucleotide phosphate oxidase
NADH	Nicotinamide-adenine dinucleotide
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NOx	Total concentration of nitrate and nitrite
NTS	Nucleus tractus solitarius
O_2	Oxygen

O_2^-	Superoxide
P	Pressure
P0	Position of the inflection point on the pressure axis
P_1	Inlet pressure
P1	P1 is the steepness of the curve at 0.75 A_{max}
P_2	Outlet pressure.
P_{2X}	purinergic receptors
P_{2X} receptors	Purinergic receptors type 2_x
P_{2X}2/3	Purinergic receptors 2/3 subtype
P_{2X}3	Purinergic receptors 3 subtypes
P_2Y	Purinergic receptors type 2Y
P_a	Mean aortic pressure
PEI	Post-exercise ischemia
PKA	Protein kinase A
PKG	Protein kinase G
PP	Pulse pressure
PSNS	Parasympathetic nervous system
PWV	Pulse wave velocity
Q	Flow
R	Radius
R	Resistance

RAAS	Renin-angiotensin-aldosterone system
RER	Respiratory exchange ratio
RPM	Revolutions per minute
RSNA	Renal sympathetic nerve activity
RVLM	Rostral ventrolateral medulla
SAD	Sinoaortic denervation
SBP	Systolic blood pressure
SHRs	Spontaneously hypertensive rats
SNA	Sympathetic nerve activity
SNS	Sympathetic nervous system
SPRINT	Systolic blood pressure intervention trial
SSNA	Skin sympathetic nerve activity
SV	Stroke volume
TPR	Total peripheral resistance
TRP_V1	Transient receptor potential cation channel subfamily V member 1
UK	United Kingdom
USA	United States of America
V	Volume
VE	Minute ventilation
VE/V_ECO_2 slope	Ventilatory efficiency slope

V_ECO_2	Volume of carbon dioxide expired
$\dot{V}O_2$ peak	Peak volume of oxygen inspired
$\dot{V}O_2$	Volume of Inspired Oxygen
W	Watts
WHO	World Health Organisation
Z_0	Aortic characteristic impedance
α1-adrenoceptors	Alpha-1 adrenoceptor
α_2-adrenoceptors	Alpha-adrenoceptor 2
β_1 adrenergic	Beta 1 adrenoreceptor

1.2 General overview

Adjustments in the autonomic nervous system are critical for the correct cardiovascular (CV) adjustments to exercise, which are mediated by the sympathetic and parasympathetic nervous system (PSNS). These adjustments are regulated by feed-forward signals from the brain (central command), feedback from skeletal muscle (the metaboreflex and the mechanoreflex) and continuous buffering by the arterial baroreceptors. In healthy individuals increased sympathetic nerve activity (SNA) during exercise causes a moderate rise in blood pressure (BP) that is principally caused by vasoconstriction in non-metabolically active areas (e.g. the liver). In the active muscle, SNA is offset in a protective process known as functional sympatholysis, where local and extrinsic factors cause vasodilation. The rise in systemic BP during exercise helps increase perfusion pressure to the active muscle. Individuals with untreated high BP (hypertension) have an exaggerated BP response to exercise, that is in part mediated by the metaboreflex. This exaggerated BP response to exercise increases the risk of adverse CV events. First line anti-hypertensive treatment reduces BP at rest, but it is unclear if adequate control of BP in people with hypertension decreases BP during exercise or during metaboreflex isolation (post-exercise ischemia (PEI)). This is important to assess because patients with treatment-controlled hypertension have an elevated CV risk when compared to normotensive controls. An exaggerated BP response to exercise may contribute to this elevated risk. This thesis is concerned with the role of the metaboreflex in the BP response to exercise in people with treated-controlled hypertension, and to assess whether reducing metaboreflex activity is associated with improvements in exercise BP in this population.

1.3 Basic blood pressure physiology

1.3.1 Basic haemodynamics

The CV system serves to provide rapid transport of oxygen (O_2), glucose, amino acids, fatty acids, vitamins, drugs, hormones and water to tissues in the body. In addition, the CV system is critically important in temperature regulation and the washout of metabolic waste products such as carbon dioxide (CO_2) (Nobrega et al., 2014). The heart provides the pump to force this blood around both the systemic and pulmonary circulation. This thesis is concerned with the systemic circulation. Cardiac output (CO) is defined as the amount of blood ejected by the right or left ventricle per minute and is derived from stroke volume (SV) (amount of blood per ejection) and heart rate (HR) (number of contractions per minute). At rest, CO is distributed relative to the metabolic rate of tissues and it increases rapidly in proportion to the metabolic demand within the body (Guyton, 1981). For example, at rest, skeletal muscle requires around 20% of O_2 consumption and receives around 20% of CO (Figure 1.1, page 83). At maximum exercise, the O_2 demand of the skeletal muscle rises and leads to a re-distribution of CO, with up to 80% being sent to the active skeletal muscle (Figure 1.1, page 83).

The principle driver of blood flow through the circulation is the gradient of the BP. BP is the force that the circulating blood exerts on the wall of the blood vessels. Ejection of the left ventricle raises aortic pressure to around 100 mmHg above atmospheric pressure, whilst pressure in the great veins is close to the atmospheric pressure. Therefore, there is a large BP gradient between the aorta and the great veins. Arterial BP is pulsatile as the left ventricle ejects blood

intermittently (systolic) with periods of relaxation (diastolic) between beats. Normal values for systemic arterial BP are ~120/80 mmHg (systolic/diastolic) above atmospheric pressure. Mean arterial pressure (MAP) is defined as the product of CO and total peripheral resistance (TPR) (Equation 1.2). BP is measured in mmHg above atmospheric pressure because BP was initially measured using a mercury column, which used atmospheric pressure as a reference or 0 mmHg. Much of our understanding about BP comes from measuring average levels of pressure using Darcys Law. Darcy's law explains the basic laws of flow (volume transferred per unit of time) of water along a rigid tube which is driven by a constant pressure: (Mayet and Hughes, 2003):

$$Q = K*(P_1-P_2) \quad \text{[Equation 1.0]}$$

Where Q = flow, K = hydraulic conductance, P_1 = inlet pressure and P_2 = outlet pressure.

Darcys Law can also be used to quantify the resistance that flow experiences when passing through a rigid tube:

$$Q = (P_1-P_2)/R \quad \text{[Equation 1.1]}$$

R = Resistance.

If we now apply Darcys Law to the systemic circulation:

MAP = CO * TPR [Equation 1.2]

MAP is the average BP during a cardiac cycle. In the aorta, the MAP is approximately half-way between systolic (maximum blood pressure during contraction of the vessels) and diastolic (minimum pressure between heart beats) blood pressure. BP is typically measured in the brachial artery and due to the narrowing of the systolic peak due to distal transmission the MAP is closer to diastolic blood pressure and is calculated as:

MAP = DBP +0.33*(SBP – DBP) [Equation 1.3]

DBP = diastolic blood pressure

SBP = systolic blood pressure

Darcy's Law suggests that for a larger resistance there will need to be a larger difference in BP to drive blood flow. Resistance is minimal in vessels such as elastic arteries due to increased diameter, compliance, caused by elastin. The highest resistance in the CV system lies in the resistance vessels (arterioles) as these vessels contain vascular smooth muscle and are innervated by sympathetic nerve fibres. The most important factor regulating BP is therefore blood flow in the resistance vessels. Poiseuilles described the resistance to flow within a vessel as:

$R = 8\eta L/\pi r^4$ [Equation 1.4]

R = resistance

η = viscosity

L = length

r = the radius

Poiseuille's Law shows the importance of the radius (to the power of 4) in determining resistance and therefore MAP (Singh et al., 2013). Combining Poiseuille's Law with Darcys Law we get:

$Q = (P_A - P_V) * \pi r^4/8\eta L$ [Equation 1.5]

The Windkessel effect explains the pulsatile nature of arterial BP. Large elastic arteries (e.g. the aorta), which contain a large amount of elastin, distend during systole and convert some of the pressure generated by the left ventricle into potential energy (Mayet and Hughes, 2003, Frank, 1990). Following left ventricular contraction, the aorta recoils and the stored potential energy is converted into pressure energy, which is the diastolic component of BP (Mayet and Hughes, 2003). This helps to dampen the fluctuation in pulse pressure (PP) (PP=SBP-DBP) during the cardiac cycle and allows continued perfusion during diastole when left ventricular ejection ceases (Mayet and Hughes, 2003). The Windkessel effect highlights the importance of arterial compliance in the circulation. The arterial pressure wave transmitted by the aorta undergoes

changes in shape throughout the circulation (Mayet and Hughes, 2003). When the heart contracts there is a reflected pressure wave back from the periphery (wave reflection) and a pressure wave that is amplified out (forward wave) towards the peripheral organs. The pressure wave travels rapidly though the circulation. The velocity of this pressure wave is increased by the stiffening of the blood vessels (Blacher et al., 1999). Bifurcations and high resistance arterioles are the major site of wave reflection in humans (Hirata et al., 2006). In healthy young adults, wave reflection doesn't influence aortic pressure or the peripheral pressure because the reflected wave returns during late diastole. This means that the reflected wave does not contribute to aortic BP, and brachial BP are larger than central (aortic) BPs (Hirata et al., 2006).

1.3.2 Control mechanisms of blood pressure

The following section outlines the basic control mechanisms of BP regulation when the human body is idle, before discussing the mechanisms mediating changes in BP during exercise in the following section. BP is mediated by changes in CO and TPR [equation 1.2 and 1.3]. Due to the importance of BP for flow, the arterial BP in the human body is tightly regulated by several short and long term mechanisms (Raven and Chapleau, 2014). Indeed, reductions in BP (hypotension) and subsequently blood flow lead to mismatches between O_2 demand and supply (Raven and Chapleau, 2014). The following section explains how BP is controlled in the short- and long-term.

1.3.2.1 Acute control of blood pressure

1.3.2.1.1 Cardiac output

CO is typically 5 L/min in a resting adult human. CO is varied by alterations in SV and HR (CO = SV*HR). A typical resting HR is 70 beats per minute and SV normally equals 70 ml/min in a resting human (Joyner and Casey, 2015). However, this value of CO is constantly changing to match the metabolic demand of the body (Joyner and Casey, 2015). For example, during maximal exercise, HR can reach 200 bpm and SV can reach 100-150 ml/min, giving a CO of ~20 L/min in a normally active individual.

1.3.2.1.2 Stroke Volume

SV is the amount of blood pumped out by the heart per beat and is calculated as end diastolic volume – end systolic volume (Guyton, 1981, Mayet and Hughes, 2003).

1.3.2.1.2.1 Preload and Afterload

SV is affected by two opposing factors; the energy of left ventricular contraction (determined by preload) and the pressure in the aorta that the left ventricle has to overcome to eject blood (afterload). Preload is related to the volume of blood in the left or right ventricle prior to contraction (end-diastolic volume). Augmented filling pressures increase the volume of blood in the ventricles during diastole. This enhances the contractile force generated during ventricular contraction via the length-tension relationship, which is known as the Frank-Starling Law of the

heart in intact hearts. When a myocyte is stretched it generates a more forceful contraction. Maximal force of the myocardium is achieved at a sarcomere length of around 2.2 µm and beyond this there is a reduction in contractile force (Guccione et al., 1997). In the intact heart at a normal end diastolic pressure, sarcomere length is normally 1.8- 2 µm (Guccione et al., 1997). Increased stretch of the ventricles augments the sensitivity of the cardiac sarcomeres to calcium (Ca^{2+}), causing increased displacement of the troponin-tropomyosin complexes which increases myosin head binding to actin and therefore contraction (Allen and Kentish, 1985). As central venous pressure increases so does preload and SV. Central venous pressure is influenced by several factors, including the skeletal muscle pump (Stewart et al., 2004), blood volume (Berger et al., 1968), gravity (Buckey et al., 1993), venoconstriction (constriction of the veins) by the sympathetic nervous system (SNS) (Martin and Charkoudian, 2005), cardiac pumping (Guyton et al., 1954) and respiration (Triedman and Saul, 1994). The increase in SV during exercise is mediated by the skeletal muscle pump (Notarius and Magder, 1996, Stewart et al., 2004), respiration (Miller et al., 2005) and venoconstriction (Martin and Charkoudian, 2005). Secondly, afterload is defined as the aortic pressure that the left ventricle must overcome to effectively eject blood (Monroe and French, 1961, Sonnenblick, 1962). More specifically, the pressure in the left ventricle must be higher than the pressure in the aorta to open the aortic valve and pump blood into the systemic circulation (aortic impedance). Afterload is the pressure that *opposes* muscle shortening. In the intact heart afterload is often related to the law of Laplace:

$$S = P * radius/2w \quad \text{[Equation 1.6]}$$

S= wall stress

P = ventricular ejection pressure

W= wall thickness

Laplace's law shows that afterload (S) is dependent on not only the pressure in the aorta, but also the radius of the ventricles and the wall thickness. There is an inverse relationship between afterload and instantaneous left ventricular SV. Elevated afterload opposes sarcomere shortening and limits ventricular SV which leads to an increased end-systolic volume (volume of blood left in the heart following contraction) (Monroe and French, 1961). Furthermore, in the healthy heart this increased end-systolic volume means that end-diastolic volume is increased. In the following beat there is an increased left ventricular contraction (Frank-Starling mechanism) to compensate for the reduced SV. During upright exercise, end-systolic volume decreases, whilst end-diastolic volume increases allowing SV to increase (Andersen and Vik-Mo, 1984, Poliner et al., 1980). This is thought to be due to an increased myocardial fibre length (Frank-Starling) as well as an increased contractile state of the heart.

1.3.2.1.3 The autonomic nervous system and the heart

CO is not only regulated by intrinsic factors related to contractile energy and thus variations in SV but is also regulated by the autonomic nervous system. Changes in the PSNS and SNS regulate both HR and SV. Under resting conditions, HR is predominantly under the control of the PSNS (Robinson et al., 1966).

Parasympathetic activity, via the vagus nerve releases acetylcholine which binds to the muscarinic 2 (M_2) receptor leading to a reduction in the formation of cyclic adenosine monophosphate (cAMP) and reduced calcium influx $[Ca^{2+}]i$ through the cell membrane. A reduction in $[Ca^{2+}]i$ causes a reduction in the speed at which the pacemaker action potential decays and also causes membrane hyperpolarisation, which leads to a reduction in HR (Harvey and Belevych, 2003, Bolter et al., 2001). Further, this also causes the opening of the cardiac muscarinic inward rectifying potassium channels (Ivanova-Nikolova et al., 1998), these channels increase potassium efflux which similar to M_2 channels leads to hyperpolarisation and a reduction in HR. The action of the vagus nerve is fast (within a beat), compared to the much slower action of the SNS. Noradrenaline released from the sympathetic nerve terminals bind to beta 1 (β_1) adrenergic receptors, which subsequently bind to the G stimulatory proteins (Gs) activating adenylate cyclase, causing elevations in cAMP (Madamanchi, 2007). cAMP then activates cAMP-dependent protein kinase A (PKA) which increases $[Ca^{2+}]i$ across the sarcolemma through the L-type Ca^{2+} channel in the heart (Madamanchi, 2007). This pathway increases cardiac contractility (enhancing SV) as well as increasing HR (Madamanchi, 2007), therefore CO is augmented. Binding of noradrenaline to β_1 adrenergic receptors also shortens the duration of systole, allowing sufficient filling time during diastole and decreases end diastolic volume due to increased ejection fraction (SV/end diastolic volume).

1.3.2.1.3.1 The baroreflex

The aortic and carotid baroreceptors are mechanically sensitive encapsulated afferent free nerve endings that lie within the aortic arch and the carotid sinus which modulate changes in BP on a beat-to-beat basis by mediating changes in efferent sympathetic nerve activity (SNA), HR, SV and TPR (Dampney, 2016, Raven et al., 2006, Walgenbach and Donald, 1983, Walgenbach and Shepherd, 1984). Research in canines has shown that the aortic and carotid baroreceptors are split into low threshold A-fibres that are myelinated, large diameter, fast conducting afferents with low thresholds and operate in the BP range of 30-90 mmHg (Dean and Seagard, 1997) and higher threshold C-fibres that are unmyelinated that operate at higher BPs of 70-140 mmHg (Dean and Seagard, 1997). When BP is increasing the blood vessel wall is stretched and the mechanically sensitive carotid sinus and aortic baroreceptors increase their firing rates via the glossopharyngeal nerve (cranial nerve IX) and the vagus nerve (cranial nerve X), respectively, that transmit to the brainstem and terminate in the nucleus tractus solitarius (NTS) (Dampney, 2016, Aicher and Randich, 1990, Berger, 1979, Davies and Kalia, 1981). The NTS operates as an integrative site for the control of the circulatory system from peripheral afferents (Potts et al., 2003). The NTS then projects to cardiac vagal sites in the nucleus ambiguus and to the caudal ventrolateral medulla (CVLM) (Dampney, 2016, Biaggioni et al., 1994, Suzuki et al., 1993, Kubo et al., 1991). Excitatory impulses into the nucleus ambiguus lead to activation of the preganglionic cardiac vagal neurones, causing a lowering of HR (Dampney, 2016, Housley et al., 1987). Simultaneously, the CVLM inhibits sympathetic premotor neurons located in the rostral ventrolateral medulla (RVLM) (Dampney, 2016, Pilowsky et al., 1994, Biaggioni et al., 1994, Li

et al., 1991, Guyenet, 2006). RVLM neurons project directly to the cholinergic preganglionic neurons in the intermediolateral cell column of the spinal cord (thoracolumbar regions (T1-L2)). The preganglionic neurons then terminate within the prevertebral ganglia or paravertebral ganglia, synapsing with the cell bodies of the postganglionic sympathetic neurons. These postganglionic sympathetic nerve fibres innervate target organs and release the co-transmitters noradrenaline, adenosine triphosphate (ATP) and neuropeptide Y (Burnstock, 2012, Burnstock, 1990). Noradrenaline and ATP bind to the alpha-1 adrenoceptor (α1-adrenoceptors) and purinergic receptors (P_{2X} receptors) in the peripheral blood vessels which leads to vasoconstriction and an elevation in arterial BP. In the vascular smooth muscle, the effects of noradrenaline on the α1-adrenoceptors are mediated by increased $[Ca^{2+}]i$ mainly through L-type Ca^{2+} channels (Guimaraes and Moura, 2001, Xiong and Sperelakis, 1995). ATP binds to the P_{2X} receptors and causes vasoconstriction by increasing $[Ca^{2+}]i$ mainly through L-type Ca^{2+} channels (Ralevic, 2015). Neuropeptide Y has a dual effect, prejunctionally it inhibits the release of noradrenaline and ATP and it also potentiates the post-junctional effects of noradrenaline and ATP (Burnstock, 1990). This simultaneous coupling of the SNS and PSNS allows maximal control over BP.

1.3.2.2 Long term blood pressure control mechanisms

1.3.2.2.1 The renin-angiotensin-aldosterone system

Firstly, the renin-angiotensin-aldosterone system (RAAS) plays a critical role in the regulation of long-term BP control (several hours/days) (Seeliger et al., 2005).

The RAAS responds to reductions in blood volume (e.g. dehydration), BP, sodium chloride, filtration flow rate and activation of the SNS that cause the juxtaglomerular apparatus in the kidneys macula densa to release renin. Renin release leads to the formation of angiotensin I from angiotensinogen which is released from the liver. Angiotensin I is then converted to angiotensin II, which is dependent on an enzyme called angiotensin converting enzyme (ACE), which is predominantly found in the capillaries of the lungs and the endothelial cells (Oppong and Hooper, 1993). Angiotensin II acts as a potent vasoconstrictor by binding to angiotensin type 1 receptor (AT_1) receptors and causes elevations in peripheral resistance (de Leeuw, 1999). Angiotensin II also boosts the activity of the sympathetic nervous by facilitating the release of noradrenaline from sympathetic terminals (Rajagopalan et al., 1996, Zimmerman et al., 2002). In addition, cardiac contractility is enhanced by the RAAS (Brasch et al., 1993). AT_1 receptor have been located in the myocardium and angiotensin II can increase cardiac contractility through its effects on $[Ca^{2+}]i$, similar to noradrenaline (De Mello and Danser, 2000).

The kidney itself also plays a critical role in long-term BP regulation (Guyton et al., 1972). An elevation in perfusion pressure in the renal artery causes an increased natriuresis, this is known as the pressure-natriuresis relationship (Aperia et al., 1971, Guyton, 1981). By lowering the concentration of sodium and the overall volume of fluid in the body, the pressure-natriuresis relationship helps to reduce CO (Frank-Starling mechanism) and BP (Guyton, 1981, Aperia et al., 1971). Angiotensin II also leads to the release of aldosterone which increases the reabsorption of sodium in exchange for potassium (Mulrow, 1999). Sodium

reabsorption from the tubular fluid draws water with it by osmosis and the overall volume of fluid in the body elevates and leads to an elevation in CO (Frank-Starling mechanism) and BP (Cowley, 1992).

AT_1 receptors have also been located on blood vessels and neurons in the NTS (Huang et al., 2003, Paton et al., 2006). An injection of angiotensin II into the NTS depresses the sympathoinhibition effects (Polson et al., 2007, Boscan et al., 2001) as well as the cardiac component of the arterial baroreflex (Paton and Kasparov, 1999, Paton et al., 2001, Casto and Phillips, 1986). Angiotensin II is unable to cross the blood-brain-barrier. Paton et al. (2006) proposed vascular neuronal signalling, which suggests that centrally and peripherally formed angiotensin II can act on the endothelium in the NTS causing the formation of nitric oxide (NO) (Paton et al., 2008). It has been proposed that NO reduces the firing rates of NTS neurons which respond to input from the arterial baroreflex, via increased release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Wang et al., 2006).

1.3.2.3 Long term blood pressure control by the baroreflex

It was initially believed that the baroreceptors had little role in the long-term control of arterial BP. Thrasher (2002) found that denervation of the aortic and one carotid sinus in dogs whilst chronically unloading the baroreceptors in the intact sinus through common carotid artery ligation caused hypertension. These findings have been confirmed in rats using more direct measurements of renal sympathetic nerve activity (RSNA) (Barrett et al., 2005, Barrett et al., 2003).

Further evidence for long term control of BP by the baroreflex came when it was shown that following sinoaortic denervation (SAD), which removes afferent baroreceptor input, sustained reduction in RSNA during angiotensin II infusion was abolished (Barrett et al., 2005). Finally, a Fos-like protein immunohistochemistry study found that dogs acutely and chronically infused with angiotensin II had increased Fos-like staining in the NTS and CVLM neurons with little change in the RVLM (Lohmeier et al., 2002). This result was expected as the sympathoinhibition of the baroreceptor in the RVLM is mediated by increased neuronal activity in the NTS and the CVLM (Lohmeier et al., 2002). This suggests that the baroreflex is important in the chronic regulation of arterial BP.

1.3.3 Local regulation of flow

Vascular tone impacts the radius of the blood vessel and therefore has a huge impact on blood flow (equation 1.4 and 1.5). When the vascular smooth muscle in the resistance vessels relaxes (vasodilation), resistance is reduced and local flow increases, conversely when resistance vessels tighten (vasoconstriction), local blood flow decreases, causing arterial BP to rise. Vascular tone is mediated by several intrinsic and extrinsic factors.

Intrinsic mechanisms include, myogenic (Meininger and Davis, 1992), endothelial secretions (Moncada and Higgs, 1993, Palmer et al., 1988) and vasoactive metabolites (Remensnyder et al., 1962). Firstly, the myogenic response is an immediate control mechanism (within seconds). When intravascular pressure in arteries and arterioles increases, at first this causes distention of the vessel which

is followed by a sustained contraction (Bayliss, 1902). In contrast, when intravascular pressure decreases vasodilation occurs (Bayliss, 1902). The myogenic response is important as it modulates basal tone (Folkow, 1962), stabilises capillary perfusion pressure (Meininger and Davis, 1992) and protects the capillaries from sustained high intravascular pressures (Meininger and Davis, 1992). Secondly, the main role of the endothelium is as a selectively permeable membrane to keep plasma and red blood cells within the blood vessels, whilst allowing the movement of different nutrients from the blood into a variety of tissues around the body. The endothelium is critically important in the regulation of basal tone. Endothelial cells express many receptors that bind vasoactive substances (e.g. purinergic receptors type 2Y (P_2Y receptors)) and also sense changes in shear stress (see Figure 1.4, page 87). A healthy endothelium responds by actively secreting vasoactive substances, including prostacyclin, endothelial-derived hyperpolarisation factor and NO which relax vascular smooth muscle to cause vasodilation (Davies, 1995). A key role for NO has been demonstrated in the regulation of endothelial mediated vasodilation (Joannides et al., 1995) and importantly in CV disease, abnormalities in the endothelium have been attributed to impaired NO bioavailability (Panza et al., 1994, Panza et al., 1993, Panza et al., 1990).

1.3.3.1 Mechanism of nitric oxide mediated vasodilation

The exact mechanism of shear stress mediated vasodilation is thought to involve changes in the deformation of the endothelial cells and deformation of the glycocalyx due to viscous drag (Heiss et al., 2015). Shear stress activates the

enzyme phosphatidylinositol-3 kinase, leading to the activation of protein kinase B (Heiss et al., 2015). Agonist binding to the endothelium cause an $[Ca^{2+}]i$ which leads to increased Ca^{2+} binding to calmodulin (Moncada and Higgs, 1993, Dimmeler et al., 1999) (Figure 1.4, page 87). Both, Ca^{2+}-calmodulin and protein kinase B enhance the activity of endothelial nitric oxide synthase (eNOS). eNOS cleaves the nitrogen group from L-arginine which combines with molecular O_2 to form NO and citrulline (Gao et al., 2007, Palmer et al., 1988). In addition, this reaction requires electrons that are released from nicotinamide-adenine dinucleotide phosphate oxidase (NAD(P)H), tetrahydrobioprein (BH_4) and flavin adenine dinucleotide as cofactors. When BH_4 is not present, the endothelium can become uncoupled and produce superoxide (O_2^-) instead of NO.

NO rapidly causes vasodilation by two mechanisms. Upon formation, NO diffuses into the blood stream (where it inhibits platelet function) and out of the vessel lumen into nearby vascular smooth muscle cells. In the smooth muscle cells, NO binds to the heme group in guanylate cyclase which catalyses the synthesis of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) (Tousoulis et al., 2012, Rapoport et al., 1983) (see Figure 1.4, page 87). Myosin light-chain kinase phosphorylates myosin light chains and leads to cross-bridge formation between the myosin head and actin filaments, causing smooth muscle contraction (Tousoulis et al., 2012). NO leads to increases in protein kinase G (PKG) and phosphorylates K^+_{ATP} channels, large conductance Ca^{2+}-activated K^+ channels, inward-rectifying K+ channels, Na+-K+ pump activity and the Ca^{2+} ATPase pumps in the plasma membrane (Thomas and Segal, 2004). Furthermore, PKG increases the Ca^{2+} and K^+ efflux from the vascular smooth

muscle cells, causing hyperpolarisation. This subsequently inhibits $[Ca^{2+}]i$ through L-type calcium channels and causes vascular smooth muscle relaxation through myosin light-chain phosphatase phosphorylation which dephosphorylates myosin light-chain kinase (see Figure 1.4, page 87) (Thomas and Segal, 2004, Jackson, 1993). Finally, NO is inactivated within a couple of seconds in the vascular smooth muscle by the presence of O_2^- anions, which are a by-product of metabolism. NO reacts with O_2^- anions to form peroxynitrite in the vascular smooth muscle and NO also binds to red blood cells due to NOs high affinity for haem.

Another determinant of blood flow is metabolic demand. Metabolic hyperaemia is an increase in organ blood flow that is associated with increased metabolic activity. This response is largely driven by a mismatch in O_2 delivery and demand which can be facilitated by factors such as reduced oxygenation of the blood, decreased perfusion to an organ or an increase in the metabolic demand of an organ (Reglin and Pries, 2014) (see Figure 1.4, page 87). This occurs during exercise and the response is known as functional hyperaemia or functional sympatholysis and plays a critical role in the CV adjustments to exercise (Remensnyder et al., 1962) (see below; section 1.4.1.2.3 *Efferent, page 37*).

1.4 The normal cardiovascular response to exercise

The previous section described the mechanisms that control BP at rest, this section moves this discussion on to mechanisms that mediate the changes in the CV system associated with exercise. The co-ordinated CV response to physical

exercise (or exertion) is probably one of the most important circulatory response for the survival of all animals. More is learnt about the regulation of the human body by studying its responses to an acute bout of stress (e.g. exercise) than when it is idle. If an acute bout of exercise is repeated on several occasions, adaptations will occur, allowing the animal to exercise for longer. This thesis is focused on the acute responses to exercise.

Physical exercise is an extremely potent stressor for the CV system and the mechanism by which BP is regulated largely depends on the mode of exercise, that being dynamic or isometric. During dynamic exercise, SNA is diffusely increased to the arterioles of all organs to cause vasoconstriction (e.g., splanchnic region and the non-active skeletal muscle), which maintains perfusion pressure into the active skeletal muscle (Remensnyder et al., 1962, Amann et al., 2011a, Amann et al., 2010, Amann et al., 2011b). A threat to perfusion pressure that occurs during exercise is the increased sweating rates which cause loss of plasma volume and sodium (Galbo, 1986). In healthy individuals alterations in glomerular filtration rate (Aldigier et al., 1993) and increases in SNA (Joles et al., 1982, Ahlborg and Lundberg, 1996) directed to the kidney act via the β-adrenoceptors to increase the release of renin and subsequently aldosterone and vasopressin which inhibit renal sodium and water excretion (Milledge and Catley, 1982), blunting loss of plasma volume and helping to maintain perfusion pressure. The release of renin appears to be modest at lower intensities of exercise in healthy humans but increases linearly with exercise intensity as SNA increases (Staessen et al., 1987, Tidgren et al., 1991). Venous return is also augmented during dynamic exercise, mostly by the skeletal muscle pump, which

increases end-diastolic volume (Casey and Hart, 2008). Increased venous return and sympathetically mediated inotropic effects on the left ventricle (reduced end-systolic volume) mediate a large change in SV (Asmussen, 1981). Vagal withdrawal and sympathetic activation of the heart lead to a chronotropic effect that together with the increased SV lead to increases in CO (Asmussen, 1981, Burton et al., 2004). Metabolically induced vasodilation within the active skeletal muscle causes a drop in TPR during exercise. The increase in CO is larger than the drop in TPR and during dynamic exercise there is a small increase in MAP (Asmussen, 1981, Burton et al., 2004) (see Figure 1.3, page 85).

In contrast, during isometric exercise, the intramuscular pressure can be so high that it compresses the blood vessels and occludes blood flow (Alam and Smirk, 1937). During isometric exercise arterial BP rises to a much greater degree compared to dynamic exercise with the aim of overcoming the compressed blood vessels to maintain adequate perfusion pressure (Kaur and Mann, 2016, Lind et al., 1964). Dynamic and static exercise are the two broad ends of the exercise spectrum and most physical activity is categorised as a combination of both, for example walking with a shopping bag or weight lifting involves both isometric and dynamic components.

1.4.1 What initiates and controls the circulatory adjustments during exercise?

It is well established that several neural pathways work together during both dynamic and isometric exercise to precisely control the CV system. More precisely, feed-forward signals from the higher brain, known as central command

(Zuntz and Geppert, 1886, Goodwin et al., 1972, Williamson et al., 2002, Williamson et al., 1995), feedback from the skeletal muscle, the exercise pressor reflex (Kaufman, 2012, Kaufman et al., 1983, Kaufman et al., 1984, Amann et al., 2011a, Amann et al., 2010, Amann and Light, 2015, Amann et al., 2009, Amann et al., 2011b) and continuous buffering from the carotid baroreflex and the aortic baroreflex (Bevegård and Shepherd, 1966a, Melcher and Donald, 1981) mediate the CV responses to exercise.

1.4.1.1 Central command

As early as 1886 Zuntz and Geppert proposed a central mechanism that activated respiratory and voluntary locomotor pathways (Zuntz and Geppert, 1886) and was later shown to also influence the CV response to exercise (Krogh and Lindhard, 1913, Krogh and Lindhard, 1917). Central command is a feed-forward mechanism originating in areas of the cerebral cortex and/or motor regions. Central command involves descending neural signals before and during exercise with parallel activation of both motor and CV control centres within the medulla (Goodwin et al., 1972). Prior to exercise, increased central command mediates the anticipatory rise in HR (McArdle et al., 1967). Animal studies have highlighted that the subthalamic locomotor region/hypothalamic motor region which includes the hypothalamus and the fields of Forel (Abrahams et al., 1960, Eldridge et al., 1981, Eldridge et al., 1985, Smith et al., 1960, Tan and Dampney, 1983, Waldrop et al., 1988, Waldrop et al., 1986), the rostral portion of the periaqueductal gray (Bandler and Carrive, 1988, Smith et al., 1960, Tan and Dampney, 1983) and also the amygdala (Hilton and Zbrozyna, 1963, al Maskati

and Zbrozyna, 1989) are important in regulating both the locomotor and the CV changes during exercise. In animals, it has been shown that central command mediates changes in parasympathetic and sympathetic outflow via input to the RVLM (Hilton et al., 1983, Waldrop and Bauer, 1989). However, an issue with the studies assessing central command in animals is that exercise is simulated via chemical or electrical stimulation of the brain regions and it is unknown if actual exercise would cause activation of the same brain pathways. A further disadvantage of the studies assessing the brain regions responsible for central command is the invasive approaches used in animal studies obviously cannot be repeated in humans during exercise.

Evidence in humans, using handgrip exercise during positron emission tomography scans and functional magnetic resonance imaging has also highlighted that the insular and anterior cingulate cortices are important in mediating central command during exercise (Critchley et al., 2000, King et al., 1999, Nowak et al., 2005, Nowak et al., 1999, Williamson et al., 2003, Williamson et al., 2002, Williamson et al., 2001). In addition, recent studies completed during deep brain stimulation in patients has furthered our knowledge of brain pathways involved in CV regulation during exercise. More specifically, stimulation of the dorsal periventricular/periaqueductal gray (Basnayake et al., 2011, Green et al., 2005, Green et al., 2007), subthalamic nucleus (Green et al., 2007), the thalamus and the substantia nigra (Thornton et al., 2002) have shown to elicit CV changes similar to exercise. Central command appears to be important in the regulation of HR prior to and at the onset of exercise in humans, as the increase in HR is too rapid to be associated with feedback from skeletal muscle (exercise pressor

reflex) (Muthalib et al., 2009). It was originally thought that central command mediates increases in HR via withdrawal of the PSNS (Mitchell et al., 1989). Additionally, following tubocurarine-induced neuromuscular blockade which reduces the contractile ability of the active skeletal muscle and increases the amount of central command needed to maintain a certain workload, the initial HR response to voluntary static exercise is maintained in humans (Iwamoto et al., 1987). The change in BP was reduced during blockade, but not fully diminished, indicating that central command can also effect BP at the onset of exercise. Exaggerated CV responses have been found with partial neuromuscular blockade during prolonged isometric handgrip exercise (Leonard et al., 1985, Mitchell et al., 1989). The HR and BP response to imagined (hypnosis) and actual handgrip are identical, suggesting that central command can be influenced by the perception of effort of exercise (Williamson et al., 2002). Plasma catecholamine levels were also elevated when central command was increased during partial neuromuscular blockade with tubocurarine during static (Pawelczyk et al., 1997) and dynamic (Galbo et al., 1987) exercise.

The impact of central command on muscle sympathetic nerve activity (MSNA) in humans has been difficult to isolate and the data is confined to handgrip exercise. Using microneurography, central command has been shown to have mixed impacts on MSNA. There is evidence to suggest no effect during low to moderate intensity handgrip exercise (Victor et al., 1989, Ray et al., 1994) whereas there is evidence to suggest that central command can increase MSNA during high-intensity handgrip exercise (Victor et al., 1995). Central command appears to be most important when feedback from skeletal muscle afferents is inhibited or

dysfunctional. For example, using local anaesthetics which cause substantial muscle weakness, reduced feedback from muscle afferents and an elevated feed-forward central command led to exaggerated CV response to cycle ergometer exercise in an attempt to maintain power output (Amann and Dempsey, 2008). However, in the absence of muscle weakness, central command appears insufficient to mediate the acute CV and cardiorespiratory response to exercise in humans. Blockade of the muscle afferents in the lower limbs at the superficial dorsal root ganglion using a selective σ-opioid agonist (lumbar intrathecal fentanyl) during moderate to heavy cycle ergometer exercise in healthy males caused substantial reductions in circulatory responses as well as ventilatory responses compared to a placebo (Amann et al., 2009, Amann et al., 2010, Amann et al., 2011a, Amann et al., 2011b). This led to reductions in perfusion pressure, arterial hypoxemia and blood flow in the active skeletal muscle which led to a reduction in the efflux of lactate increasing locomotor muscle fatigue (Amann et al., 2009, Amann et al., 2010, Amann et al., 2011a, Amann et al., 2011b).

1.4.1.2 The exercise pressor reflex

1.4.1.2.1 Afferent

The peripheral mechanisms mediating the CV response to exercise have been studied in greater depth relative to central command, perhaps because they are easier to assess in humans. The exercise pressor reflex is a feedback system from the active skeletal muscle that contributes towards CV regulation during exercise. As early as 1894, Johansson (Johansson, 1894) suggested that

feedback from the skeletal muscle was capable of causing adjustments in the CV system during exercise. In 1937, Alam and Smirk proved the existence of a peripheral mechanism from the skeletal muscle in humans (Alam and Smirk, 1937). They found that occluding blood flow in the thighs at the onset of calf-raising exercise for ~12 minutes following exercise led to BP remaining elevated compared to free flow exercise (Alam and Smirk, 1937). Similar results were found using forearm exercise (Alam and Smirk, 1937). The authors postulated that maintenance of BP following exercise with occlusion was due to metabolites produced during contraction that were trapped in the exercising forearm (Alam and Smirk, 1937). This later became known as post-exercise ischemia (PEI).

Within the skeletal muscle there are four different groups of afferents, large diameter group I and II afferent fibres which are thickly myelinated and smaller diameter thinly myelinated group III (Aδ) and unmyelinated group IV (C) fibres (Boyd and Kalu, 1979, Hunt, 1954, McCloskey and Mitchell, 1972). An important study in the area came from Coote et al. (1971) when it was found that tetanic contraction of the hindlimb elicited by stimulating ventral roots L6-S1 in anaesthetised cats caused an increase in arterial BP and a smaller rise in HR (Coote et al., 1971). Furthermore, when the dorsal roots L6-S1 were sectioned ventral root stimulation (L6-S1) did not cause a pressor response (Coote et al., 1971). Another important finding from this study was that sectioning the articular nerves to the knee and ankle joints had no effect on the pressor response to ventral root stimulation (L6-S1) (Coote et al. 1971). These results suggested that the pressor response originates from the skeletal muscle. Further, using anodal block to inhibit group I and II fibres into the dorsal root, and anaesthetic blocks to

abolish group III and group IV fibres into the dorsal root, McCloskey and Mitchell (1972) found that the larger fibres (type I and II) have little influence on the CV alterations during exercise (McCloskey and Mitchell, 1972). The CV reflex from the skeletal muscle was mediated by the group III and IV afferents (McCloskey and Mitchell, 1972, Coote et al., 1971). The group III receptors have a conduction velocity of between 2.5 and 30 m/s in cats and between 1.6 and 10 m/s in rats (Kaufman et al., 1983, Kaufman et al., 1984, Mense and Stahnke, 1983, Pickar et al., 1994, Stone and Kaufman, 2015). This large range of conduction velocities is due to a large proportion of the group III afferents being rapidly stimulated by mechanical stimuli, whereas a smaller portion of the group III afferents are polymodal and respond to metabolic stimuli (Kaufman et al., 1983). The majority of group III afferents are quickly activated at the onset of isometric contraction and fire at a reduced rate during continued isometric contraction (Kaufman et al., 1983, Cui et al., 2007, Cui et al., 2008b). Using the same protocol, the group IV receptors have conduction velocities of less than 2.5 m/s in cats and 1.6 m/s in rats and respond mainly to metabolic by-products of contraction and are known as metaboreceptors (Kaufman et al., 1983, Kaufman et al., 1984, Mense and Stahnke, 1983, Stone and Kaufman, 2015). The group IV afferents begin firing after around 5-30 seconds of isometric contraction and their firing rate gradually increases with prolonged exercise, with further increments during ischemic exercise (Kaufman et al., 1983). PEI causes a higher percentage of group IV afferents to become activated compared to group III afferents (Kaufman et al., 1984), and PEI has since been used as a marker of metaboreflex activity which is independent of central command and the mechanoreflex. To assess the effects of dynamic exercise, the posterior hypothalamus near the Fields of Forel or the

midbrain near the uniform nucleus in decerebrate cats were stimulated whilst afferents signals were recorded from group III and IV afferents (Pickar et al., 1994, Adreani et al., 1997). These studies found that the group III mechanoreceptors discharge in time with the step cycle (Adreani et al., 1997, Pickar et al., 1994) and that the group IV metaboreceptor afferents are activated at very low levels of dynamic exercise (Adreani et al., 1997). The results of this study questioned the previous view that the metaboreflex was only stimulated by ischemic conditions (Kaufman et al., 1983).

It is proposed that the exercise pressor reflex has two main roles during exercise. Firstly, it sends an error signal to the central nervous system when there is a mismatch between O_2 supply and demand (McCloskey and Mitchell, 1972, Kaufman et al., 1983, Kaufman et al., 1984, Alam and Smirk, 1937). Secondly, it is activated during light exercise intensities and plays a key role in mediating the CV adaptations to an acute bout of exercise.

The free endings of the group III afferents originate in the connective tissue (collagen), the adventitia of the arteries and veins, tendon tissue and between the skeletal muscle fibrocytes (Andres et al., 1985, Stacey, 1969). The free endings of the group IV afferents originate in the adventitia of the arterioles and capillaries below the muscle fascia but are also located in the adventitia of the veins/venules of skeletal muscle and the adventitia of lymphocytes (Andres et al., 1985, Stacey, 1969). Thus, the metaboreceptors are in an ideal position to relay metabolic disturbances between the blood vessels and the surrounding tissue to the

brainstem during exercise (Molliver et al., 2005, Andres et al., 1985). In addition some of the free endings of the group IV afferents have been located in collagen and tendon tissue (Stacey, 1969) suggesting mechanoreceptor function.

Several chemical mediators have been highlighted that activate the group IV metaboreceptors during exercise in animal models including endogenous ATP (McCord et al., 2010, Kindig et al., 2006, Kindig et al., 2007), bradykinin (Kaufman et al., 1983) and prostaglandins (Rotto et al., 1990). In addition, the exogenous substance capsaicin, which is a chemical found in chili peppers, has been shown to activate the group IV metaboreceptors (Kaufman et al., 1983, Smith et al., 2005b, Michael and Priestley, 1999). Important roles for the acid sensing ion channel (ASIC), in particular ASIC3, the purinergic receptors 2/3 and 3 subtypes ($P_{2X}2/3$, $P_{2X}3$) and transient receptor potential cation channel subfamily V member 1 (TRP_V1) have been reported (Light et al., 2008, Jankowski et al., 2013, Pollak et al., 2014, Hanna and Kaufman, 2003). It was found that all of the metabolites studied (protons, ATP and lactate) were more effective than 1 or 2 metabolites in activating the afferent neurons, suggesting a synergistic effect of metabolites on the group IV metaboreceptors (Light et al., 2008). Light et al (2008) also indicated that there are potentially two populations of group III/IV afferents in the skeletal muscle. More specifically, one population respond to low levels of metabolites, likely representing non-noxious, free flow dynamic exercise, contributing to the autonomic responses (Light et al., 2008, Jankowski et al., 2013). The other population are higher threshold receptors and respond to ischaemic and noxious stimuli, likely playing a role in pain responses during exercise (Light et al., 2008, Jankowski et al., 2013). Injections of a concentration

of the metabolite mixture (protons, lactate and ATP) into the fascia of the abductor pollicis brevis muscle of the thumb in humans caused feelings of fatigue, similar to moderate-high intensity exercise and higher metabolite concentrations caused feelings of pain, similar to ischaemic exercise (Pollak et al., 2014).

Gadolinium, an antagonist of mechano-gated receptors, reduced the RSNA and BP response to static contraction (Kim et al., 2007) and passive tendon stretch (a purely mechanoreceptor stimulus) (Hayes et al., 2009), but had no effect on group IV afferents in decerebrated cats (Hayes et al., 2009). Gadolinium is a non-specific antagonist of multiple mechano-gated channels and has not enabled the identification of proteins that enable the activation of the mechanoreceptors during exercise (Maingret et al., 2000, Coste et al., 2010). Recently, in decerebrate rats, a toxin of the tarantula spider Grammostola spatula, also known as GsMTx4, a specific antagonist of Piezo1 and Piezo2 mechano-gated channels was shown to reduce BP and RSNA during both tendon stretch and intermittent contractions (mechanoreflex stimuluses) of the hindlimb of decerebrated, unanaesthetised Sprague-Dawley rats (Copp et al., 2016). As previously mentioned, some of the group III mechanoreceptor afferents are polymodal, and respond to metabolites in their receptive field. Local cyclooxygenase inhibition by infusion of ketorolac reduces the BP and MSNA response to muscle stretch during PEI in humans (Cui et al., 2007, Cui et al., 2008b). Suggesting that prostaglandins may play a role in sensitizing the group III mechanoreceptors during exercise, especially under ischemic conditions (Cui et al., 2008b).

1.4.1.2.2 Central pathways

The group III and IV afferents synapse at the superficial dorsal root, specifically Rexed's laminae I, II (inner medial aspect), and V (Kalia et al., 1981, Mense and Craig, 1988, Wilson et al., 2002, Panneton et al., 2005). Several neurotransmitters and neuromodulators are involved in the synapse at the superficial dorsal root, including neuropeptides such as substance P (Wilson et al., 1993a, Wilson et al., 1993b) and somatostatin (Wilson et al., 1992), excitatory amino acid neurotransmitters, including glutamate (Hand et al., 1996a) and aspartate (Hand et al., 1996a, Hand et al., 1996b) and the neurotransmitter GABA (Wang et al., 2013). Several modulators have also been studied that can mediate the sensory transmission within the superficial dorsal root including, alpha-adrenoceptor 2 (α_2-adrenoceptors) (Ally et al., 1994), P_{2X} receptors (Li et al., 2009), the serotonergic 1_A receptors (Nobrega et al., 1995), neuronal nitric oxide synthase (nNOS) (Li and Mitchell, 2002) and σ-opiate receptors (Hill and Kaufman, 1990, Amann et al., 2011a, Amann et al., 2010, Amann et al., 2009, Amann et al., 2011b). Anterograde tracing studies have shown that the main supraspinal targets for the lamina I include the CVLM, the NTS, the lateral parabrachial area, the periaqueductal grey matter and the thalamus (Gauriau and Bernard, 2004, Todd, 2010). Lamina II mainly receive information from group III and IV afferents and act as modulator, passing this information onto lamina III and IV (Lima and Coimbra, 1991). However, lamina II have also been shown to project to the CVLM (Lima and Coimbra, 1991). Lamina V neurones mainly project to the thalamus (D'Mello and Dickenson, 2008). Key central areas involved in processing of the exercise pressor reflex include activation of the dorsal and the rostral periaqueductal gray (Basnayake et al., 2011, Li and

Mitchell, 2000), NTS (Potts et al., 2002, Kalia et al., 1981, Toney and Mifflin, 1994), and the RVLM (Iwamoto and Kaufman, 1987, Iwamoto et al., 1982). Additionally, cells within in the nucleus ambiguus are inhibited by the exercise pressor reflex (Iwamoto and Kaufman, 1987). Using electrophysiological techniques, it has been shown that NTS neurons are directly excited by mechanical and metabolic stimuli within the skeletal muscle and the NTS appears to be a critical integration site for the exercise pressor reflex (Person, 1989, Toney and Mifflin, 2000).

1.4.1.2.3 Efferent

The exercise pressor reflex mediates the CV responses to exercise by increasing SNA whilst simultaneously causing withdrawal of PSNA (Mark et al., 1985). A critical physiological response during exercise is the ability of the vasculature perfusing the active skeletal muscle to vasodilate even when SNA is elevated (Remensnyder et al., 1962). Functional sympatholysis is not an all-or nothing phenomenon and sympathetic vasoconstriction is gradually offset as exercise intensity increases in humans (Tschakovsky et al., 2002). Using intravital microscopy it has been found that in the hamster retractor muscle functional sympatholysis is least apparent in feed arteries that readily constrict during exercise when SNA increases (VanTeeffelen and Segal, 2003). In the rat cremaster muscle, the proximal arterioles remain susceptible to sympathetic vasoconstriction, whereas in the distal arterioles functional sympatholysis occurs (VanTeeffelen and Segal, 2003, Anderson and Faber, 1991). In rat cremaster muscle (McGillivray-Anderson and Faber, 1990, Anderson and Faber, 1991,

Faber, 1988) there is an increased density of α1-adrenoceptors on the feed arteries and larger proximal arterioles; whereas, α_2-adrenoceptors are more prominent on the distal arterioles. However, the cremaster muscle acts to stabilise the testes, with no skeletal attachments (Moore et al., 2010). In the gluteus maximus of the mouse, the α_2-adrenoceptors are more reactive in the proximal arterioles and α1-adrenoceptors in the distal arterioles (Moore et al., 2010). In addition, a non-selective β-agonist (isoproterenol) caused near maximal dilation in both proximal and distal arterioles (Moore et al., 2010). However, the role of the β-adrenoceptors remains unclear in the skeletal muscle. Although, not fully understood, the release of vasodilatory substances from the skeletal muscle (Tu et al., 2010), endothelial cells (Segal, 2016, Hearon et al., 2016) and/or release of vasodilators from erythrocytes (Mortensen et al., 2011) can attenuate post-junctional adrenoceptor mediated vasoconstriction during exercise causing sympatholysis (Anderson and Faber, 1991, Wray et al., 2004).

An initial increase in SNA during exercise results in an acute vasoconstrictor response in the arterioles and a reduction in O_2 delivery, this stimulates an vasodilatory signal upstream which initiates vasodilation and offsets the vasoconstriction (Roy and Secomb, 2014). This phenomenon is known as sympathetic escape (Roy and Secomb, 2014). In feed arteries, sympathetic vasoconstriction overcomes any metabolic vasodilation as compared to distal arterioles where a much larger metabolic stimulus causes upstream vasodilation (Roy and Secomb, 2014).

In healthy animal models, including rats and hamster, several candidate mechanisms have been studied, including, potassium ions (Jackson, 2000, Quayle et al., 1997), ATP-sensitive K^+ channels (Jackson, 1993, Thomas et al., 1997a), tissue hypoxia (Roy and Secomb, 2014, Granger et al., 1976), prostaglandins (Kilbom and Wennmalm, 1976) and NO (Thomas and Victor, 1998, Thomas et al., 2003). To assess the metabolites responsible for functional sympatholysis in humans, the majority of studies have aimed to mimic the process by systemic sympathetic stimulation using exercise alone or blocking the effect of locally administered tyramine (used to increase noradrenaline release) during exercise (Dinenno and Joyner, 2004). Using these techniques, it has been shown that NO (Chavoshan et al., 2002, Mortensen et al., 2009a, Nyberg et al., 2012), ATP (Mortensen et al., 2009a, Mortensen et al., 2009b, Rosenmeier et al., 2004) and prostaglandins (Dinenno and Joyner, 2004, Mortensen et al., 2007, Schrage et al., 2004) appear to play a key role in mediating functional sympatholysis in healthy humans (Figure 1.4, page 87). Exactly how these substances mediate functional sympatholysis is unknown. One possible mechanism may be increased calcium and potassium efflux from the vascular smooth muscle, which would lead to vascular smooth muscle hyperpolarisation and a closure of the L-type calcium channels (Jackson, 2000) (see Figure 1.4, page 87). If NOS or cyclooxygenase are inhibited using N-nitro-L-arginine methyl ester (L-NAME) or ketorolac functional sympatholysis will still occur in healthy humans (Dinenno and Joyner, 2004, Mortensen et al., 2007, Schrage et al., 2004). This suggests redundancy in the mechanisms that mediate functional sympatholysis in healthy humans. However, combined blockade of NOS and

cyclooxygenase is sufficient to diminish functional sympatholysis (Dinenno and Joyner, 2004).

1.4.1.2.4 The arterial baroreflex during exercise

Originally, it was thought that the arterial baroreflex was 'shut-off' during exercise, as there are simultaneous increases in BP and HR. However, Bevegård and Shepherd (1966b) found that the arterial baroreflex continued to operate in humans and is reset during supine exercise to operate at higher BPs. This was evidenced by the use of neck suction (which increases baroreceptor stimulation) during exercise which led to a reduction in HR and a drop in BP (Bevegård and Shepherd, 1966b). The opposite occurred with the use of neck pressure (which reduces baroreceptor stimulation). The arterial baroreflex is continuously reset as exercise intensity increases (Ogoh et al., 2007). It has also been shown that in humans the contribution of peripheral vascular resistance to changes in BP increases as exercise intensity increases and the contribution of CO is reduced (Ogoh et al., 2003). Although not fully understood, the arterial baroreflex appears to be reset by a combination of central command (Gallagher et al., 2001a, McIlveen et al., 2001) and the exercise pressor reflex (Iellamo et al., 1997, Gallagher et al., 2001b, Fisher et al., 2008, Potts et al., 2003). In addition, the arterial baroreflex buffers the changes in SNA and BP caused by activation of the exercise pressor reflex during exercise (Waldrop and Mitchell, 1985, Kim et al., 2005, Smith et al., 2006).

1.5 Hypertension

Up to this point, the CV response to exercise has been summarised in healthy humans and animals. The CV response to exercise is altered in CV disease and provides information regarding the pathophysiology of the disease. The remainder of this Chapter is focused on the CV response to exercise in animal models and humans with hypertension.

Essential hypertension is defined as hypertension that does not have a known cause. This thesis is only concerned with essential hypertension and not secondary hypertension induced by endocrine disorders that cause hypertension or pregnancy induced hypertension. In essential hypertension, BP is chronically elevated at rest and is associated with abnormalities in the CV system at both rest and during exercise.

Hypertension is the leading modifiable risk factor that contributes towards CV disease; it affects 31% of the global population (Mills et al., 2016) and is responsible for around 10.4 million deaths per year (Forouzanfar et al., 2015, Forouzanfar et al., 2016). Of concern, only 10% of hypertensive patients in England have BP under control (<140/90 mmHg) (Fuller et al., 2017). However, a problem with above mentioned studies is the reliance on clinic/office BP measurements. It has been shown that white-coat hypertension, defined as office/clinic BPs >140/90 mmHg with normal 24-hour ambulatory blood pressure monitoring (ABPM) results (<130/80 mmHg) has a high prevalence of 15-30% (Franklin et al., 2013, O'Brien et al., 2013). According to the European Society of

Hypertension, hypertension should be confirmed by the use of 24-hour ABPM (O'Brien et al., 2001, O'Brien et al., 2000). As the population continues to expand, the annual global cost of hypertension could reach £1 trillion (Gaziano et al., 2009). The most recent Public Health England statistics suggest that 28% of adults have high BP and 12% of adults have untreated hypertension which costs the National Health Service (NHS) a staggering £2.1 billion every year (Fuller et al., 2017). Public Health England has calculated that if the national average for BP drops by 5 mmHg it will save the NHS £850 million over the next 10 years (Fuller et al., 2017).

There is a continuous risk of mortality from stroke, ischemic heart disease and all-vascular causes with resting BP above 115/75 mmHg (Lewington et al., 2002). In-line with this data, a recent Lancet meta-analysis found that the relative risk for major CV events, stroke, heart failure and all-cause mortality were proportional to the reduction in BP through anti-hypertensive medications (Ettehad et al., 2016). Finally, the systolic blood pressure intervention trial (SPRINT) found that among patients with hypertension, treating and controlling SBP to <120mmHg, compared to <140mmHg resulted in fewer fatal and nonfatal major CV events and total mortality over a median follow-up of 3.26 years (Wright et al., 2015). These studies highlight the clear importance of lowering BP in hypertension. The SPRINT study resulted in the reclassification of stage 1 hypertension, which was defined as SBP between 130-139 mmHg and DBP between 80-89 mmHg being redefined as >120/80 mmHg in the United States of America (USA) (Whelton et al., 2017).

1.5.1 The pathophysiology of hypertension

It is well established that large artery (aortic) stiffness increases with ageing (Najjar et al., 2005) and arterial stiffness is further enhanced in hypertension (Koivistoinen et al., 2017, Laurent et al., 2001). In hypertension, there is an increased wave reflection from the periphery, which occurs early in systole and contributes to the aortic BP and causes aortic BP to rise to a similar level as brachial BP (Hirata et al., 2006). This increases the load on the left ventricle, reduces ejection fraction and augments the myocardial O_2 requirements (Laurent et al., 2001). Both males and females with hypertension have an increased left ventricular mass. However, chronic hypertension is more likely to lead to concentric hypertrophy (increased left ventricular wall thickness) in women and eccentric hypertrophy (dilation of the left ventricle) in males (Vriz et al., 1997, Krumholz et al., 1993). Concentric hypertrophy in females is associated with increased thickening of the ventricular wall, which normalises the stress on the cardiac myocytes without changing the left ventricular cavity size (LaPlaces Law; Equation 1.6) (Krumholz et al., 1993). Eventually, the increased diffusion distance into the myocardium (increased wall thickness) leads to hypoxia and subsequently necrosis and apoptosis of the cardiac myocytes. In males, eccentric hypertrophy is associated with normal wall thickness and an increase in the left ventricular diameter which can lead to an elevated wall stress and left ventricle failure (decompensated hypertrophy) (LaPlaces Law; Equation 1.6) (Krumholz et al., 1993).

Similarly, in the periphery, the increased load imparted on the arterial wall by chronic vasoconstriction and elevations in arterial BP increases wall tension (Mayet and Hughes, 2003). Tensile stress can be normalised by increases in wall thickness and/or reductions in the diameter of the arterial lumen (Mayet and Hughes, 2003). Increased wall thickness in hypertension is mediated by smooth muscle cell hyperplasia and increased collagen-to-elastin ratio, which leads to augmented media-to-lumen ratios (Mulvany et al., 1985). Small artery remodelling is the first sign of end organ damage that appears in hypertension, prior to endothelial dysfunction and left ventricular hypertrophy (Park and Schiffrin, 2001). It is well documented that the endothelium is impaired in individuals with hypertension, leading to reductions in NO bioavailability (Panza et al., 1994, Panza et al., 1990). The Framingham Heart study found that reduced flow-mediated dilatation (FMD, a marker of endothelial dysfunction) was positively related to the severity of hypertension (Benjamin et al., 2004). Two of the most important drivers of endothelial dysfunction in hypertension are oxidative stress and vascular inflammation. Oxidative stress related to endothelial dysfunction appears to be mediated by increases in NAD(P)H, which uses nicotinamide-adenine dinucleotide (NADH)/NAD(P)H as an electron donor to reduce O_2 to O_2^- anions (Paravicini and Touyz, 2006). Chronic exposure of isolated carotid arteries (from mice) to high intraluminal pressures leads to elevated levels of O_2^- and NAD(P)H oxidase, which are related to reductions in endothelium-dependent vasodilation from acetylcholine (Vecchione et al., 2009). In addition, elevated levels of ROS (such as hydrogen peroxide, peroxynitrite and O_2^-) produced from the mitochondria can also cause endothelial dysfunction (Doughan et al., 2008). In regard to inflammation, a reduction in the level of

endothelial progenitor cells (EPC's) is related to impaired endothelial function in the presence of complement factor 3 (C3) (Hill et al., 2003, Giannotti et al., 2010). Endothelial dysfunction and altered large and small artery function leads to reductions in the capabilities of the arteries and arterioles to dilate in hypertensives. This leads to elevated vascular resistance in nearly every organ, including the kidney. SV appears to be normal or slightly reduced in essential hypertension, due to elevated afterload (Kahan and Bergfeldt, 2005, Safar et al., 1976). Heart rate is typically higher in patients with essential hypertension, which likely reflects the heightened sympathetic tone associated with essential hypertension (see section *1.5.1.1. The sympathetic nervous system and hypertension*) (Morcet et al., 1999). A study of 101 patients with hypertension and 101 normotensives found that CO remained normal in essential hypertension (Safar et al., 1976).

1.5.1.1 The sympathetic nervous system and hypertension

It is well established that patients with hypertension have higher levels of SNA as measured by microneurography and noradrenaline spillover (Judy et al., 1979, Abboud, 1982, Warnert et al., 2016, Grassi and Ram, 2016, Esler, 2000). In addition, it has been shown that the level of SNA increases with the degree of hypertension and end-organ damage (Grassi et al., 1998a, Smith et al., 2004). These findings suggest a neurogenic component to the development and progression of hypertension (Mancia et al., 1999). Noradrenaline spillover is increased in the renal, cerebral and the coronary circulation, but normal in splanchnic and pulmonary regions (Esler et al., 1989). Skin sympathetic nerve

activity (SSNA) (measured via microneurography) is normal in hypertension but elevated to the vascular smooth muscle beds in essential hypertension (MSNA) (Grassi, 2009), highlighting that SNA over activity in hypertension is nonuniformly distributed around the body. Elevated α_1 adrenoceptor activation causes an increase in NAD(P)H activation (Amin et al., 2001). α_1 adrenoceptor activation and O_2^- activate a cascade of signalling pathways, including mitogen-activated protein kinases (MAPKs), L-type Ca^{2+} channels, tyrosine kinases and redox sensitive transcription pathways which lead to vascular smooth muscle cell growth and an increase in extracellular proteins, including collagen and fibronectin (Montezano and Touyz, 2014, Amin et al., 2001, Shi et al., 2006). In addition, bone mineral loss is augmented in hypertension due to higher levels of SNA (Cappuccio et al., 1999). Therefore, excessive SNA underlies some of the end organ damage associated with hypertension (Grassi and Ram, 2016, Grassi et al., 2015), including the elevation in vascular hypertrophy and remodelling (Chalothorn et al., 2005), the triggering of proatherogenic vascular effects (Zukowska, 2005), increased wave reflection (Hart et al., 2013), elevated large artery stiffness (Delacretaz et al., 2001) and augmented cardiac hypertrophy (Strand et al., 2006, Sen et al., 1974).

Exactly what stimulates heightened SNA in hypertension is still unclear. There is considerable evidence that afferent nerve fibre input from different organs is involved. It is well established that the arterial baroreflex is reset in patients with hypertension (Somers et al., 1991) and animal models (Minami et al., 2003) to operate at higher levels of BP. Furthermore, using the modified Oxford protocol, it has been demonstrated that in essential hypertension, the arterial baroreflex

partially loses its ability to control HR but maintains its ability to modulate the BP and MSNA relative to normotension (Grassi et al., 1998a). Similar findings have been found in spontaneously hypertensive rats (SHRs) (Judy and Farrell, 1979). Elevated SNA in hypertension is also facilitated by the peripheral chemoreceptors which detect hypoxia and hypercapnia, resulting in increased SNA, BP and hyperventilation (Trzebski, 1992, Abdala et al., 2012, McBryde et al., 2013). In addition, cerebral hypoperfusion may also mediate increases in SNA, the "selfish brain" acts to increase SNA and BP to maintain its own perfusion (Warnert et al., 2016). A key modulator of afferent input is the NTS and the RVLM. Centrally derived NO, produced from endothelial, neuronal or inducible isoforms of NOS decreases sympathoexcitation in the NTS and the RVLM (Paton et al., 2001, Ruggiero et al., 1996). Elevated ROS (e.g. O_2^- anions) in hypertension are known to scavenge NO both in the brainstem, causing less inhibition of SNA in hypertension (Hirooka, 2008, Hirooka et al., 2006).

1.5.1.2 The RAAS in hypertension

The RAAS is overactive in SHRs (Kobori et al., 2005) and in patients with essential hypertension, owing to alterations in sodium reabsorption and increased SNA. The RAAS contributes to the development and progression of hypertension through an increase in circulating angiotensin II and aldosterone (Admiraal et al., 1990, Freis et al., 1983). Similar to α_1-adrenoceptor activation, elevated angiotensin II levels increase NAD(P)H oxidase (Paravicini and Touyz, 2006). NAD(P)H mediated increases in superoxide anions are known to involve angiotensin II binding to the AT_1 receptor in both the vascular smooth muscle and

brainstem (Rajagopalan et al., 1996, Zimmerman et al., 2002). This mediates end-organ damage by activating MAPKs, tyrosine kinases and redox sensitive transcription pathways which lead to vascular smooth muscle cell growth and an increase in extracellular proteins, (collagen and fibronectin). Increased renal vascular resistance in hypertension is also further enhanced by angiotensin II elevations, along with sympathetic stimulation. In addition, an increase in renal resistance mediated by RSNA and elevated angiotensin II shifts the pressure-natriuresis curve upwards in hypertension (Hall, 2003, Hall, 1986). Natriuresis continues at the same rate in hypertension, but a higher pressure is needed to do so (Hall, 2003). Interestingly, around 25% of hypertensive individuals have normal activity of the RAAS. This highlights that hypertension is likely a disease with multifactorial causes.

1.5.1.3 Current antihypertensive medications

Various treatments for hypertension have been developed, most of which target the peripheral vasculature with the aim of reducing vascular tone. Anti-hypertensive medications have been developed to target the RAAS system and reduce resting BP, including, ACE inhibitors (e.g. ramipril) (Kumar Chhabra et al., 2013), AT_1 receptor blockers (e.g. valsartan) (Abraham et al., 2011), renin inhibitors (e.g. Aliskiren) (Fu et al., 2017) and aldosterone antagonists (e.g. spironolactone) (Chapman et al., 2007). The dihydropyridine class of L-type calcium channel antagonists (e.g., amlodipine) are also given to patients with hypertension. This class of drugs bind to L-type calcium channels in the heart, causing negative inotropy and on the smooth muscle, which decrease TPR and

BP (Hirooka et al., 2006, Verdecchia et al., 2005, Xiong and Sperelakis, 1995). In addition, thiazide-like diuretics, such as indapamide reduce water and sodium, improve vascular tone and reduce arterial pressure (Campbell, 1983, Roush et al., 2015). Diuretics are generally only given to hypertensive patients who cannot tolerate calcium channel antagonists. These treatments for hypertension are regarded as the first line treatments according to the National Institute for Health and Care Excellence (NICE) guidelines 2011 because these medications produce improved survival rates. β-blockers were once regarded as first-line treatment for hypertension. However, the use of β-blockers is associated with poorer outcomes (stroke) when compared to other anti-hypertensive medications (De Caterina and Leone, 2010). In addition, they are associated with adverse side effects, such as exercise intolerance (De Caterina and Leone, 2010). Similarly, α-blockers are effective at lowering BP in patients with hypertension but are not as effective as other antihypertensive medications in reducing heart failure and stroke (Messerli, 2000). Of concern, treatment with a single anti-hypertensive medication only reduces BP in around 50-60% of patients, highlighting the heterogeneity of the development and progression of hypertension (Materson et al., 1993).

Despite adequate control of BP, individuals with treated-controlled hypertension have an increased risk of CV disease, stroke and total mortality compared to normotensive individuals (Almgren et al., 2005, Lawlor et al., 2011, Brown et al., 2013). The mechanisms mediating this remain unclear, but it highlights an underlying pathology in hypertension that is not currently being treated.

The effects of the first line anti-hypertensive medications on the activity of the SNS remain highly controversial (Del Colle et al., 2007) and not completely understood. Indeed, reductions in BP with chronic use of ACE inhibitors (Grassi et al., 1998b), AT_1 receptor antagonists, (Krum et al., 2006, Fu et al., 2017, Fu et al., 2005), thiazide-like diuretics (Fu et al., 2005) and short and long acting L-type calcium channel antagonists (Noll et al., 1998, Grossman and Messerli, 1998) occur alongside increased SNA, possibly due to unloading of the baroreflex (Fu et al., 2005). In addition, Spironolactone, despite effectively lowering BP has been shown to have no effect on resting MSNA in previously untreated hypertensive patients after 3 months of use (Menon et al., 2009). It has also been shown that patients with treatment-controlled hypertension have a similar basal MSNA level when compared to patients with untreated hypertension (Materson et al., 1993, Warnert et al., 2016). Moxonidine and clonidine are centrally acting antihypertensive drugs that activate α_2/imidazoline receptors in the NTS and RVLM and decrease MSNA (Hausberg et al., 2010). However, centrally acting hypertensives are associated with unfavourable side effects such as dry mouth, impotency and sedation and are therefore not routinely given to treat hypertension. Anti-hypertensive medications have been shown to have mixed effects on bone mineral loss in hypertension (Ghosh and Majumdar, 2014). Antihypertensive medication may not lower SNA, but it does reduce mortality compared to untreated hypertension. What is concerning is that mortality is still elevated when compared to normotensives.

An alternative treatment strategy for hypertension is CV exercise training. Physical inactivity constitutes a key risk factor of developing hypertension and

several meta-analysis have concluded that increasing aerobic exercise frequency decreases resting SBP and DBP (Whelton et al., 2002) and also decreases in CV and all-cause mortality (Rossi et al., 2012) in patients with hypertension. The recent Public Health England statistics revealed that only 66% of males and 58% of females meet the aerobic activity guidelines set by the World Health Organisation (WHO) (Fuller et al., 2017) which currently suggest 150 minutes of moderate activity or 75 minutes of high intensity exercise each week (Kikuchi et al., 2018, Fagard, 2012). Importantly, a prospective cohort study showed that meeting these guidelines was associated with reduced all-cause mortality (Kikuchi et al., 2018). In addition, objective measurements of fitness (e.g., peak volume of O_2 uptake test ($\dot{V}O_2$ peak)) are inversely related to CV events/mortality and all-cause mortality in observational studies, even after adjusting for resting SBP (Pardaens et al., 1996, McAuley et al., 2009). Physical exercise has been shown to improve several markers involved in the pathophysiology of hypertension, including lowering MSNA (Sinoway et al., 1996, Laterza et al., 2007), enhancing endothelial function (Di Francescomarino et al., 2009), increasing baroreflex sensitivity (Somers et al., 1991), improving insulin sensitivity (Stewart, 2002) and a reduction in body fat mass (Palatini et al., 1994).

While consistent evidence suggests that aerobic fitness and dynamic exercise reduce resting BP in patients with hypertension, there is a cause for concern due to stroke events. Untreated *sedentary* hypertensive individuals have an exaggerated rise in SBP during acute exercise. Studies show that BP rises excessively during low-intensity isometric handgrip at 30-50% maximal voluntary contraction (MVC) (Greaney et al., 2015a, Greaney et al., 2014, Aoki et al., 1983,

Delaney et al., 2010, Choi et al., 2013, Brorson et al., 1978), during low-intensity dynamic exercise (Seguro et al., 1991, Barbosa et al., 2016, Brorson et al., 1978) and maximal dynamic exercise (Kokkinos et al., 2002) testing.

An exaggerated BP response to exercise represents an important marker for future development of essential hypertension in both young and older normotensives (Berger et al., 2015, Holmqvist et al., 2012) and pre-hypertensive individuals (Manolio et al., 1994, Tsumura et al., 2002, Singh et al., 1999, Miyai et al., 2000). The exact mechanism hasn't been clarified and it is unclear whether the structure of the resistance vessels and/or the activation of the SNS or RAAS during exercise is changed before the development of hypertension. In addition, family history of hypertension is associated with an increased risk of developing hypertension as well as an exaggerated BP and MSNA response to exercise (Greaney et al., 2015b, Hunt et al., 1986, Molineux and Steptoe, 1988). Although only speculative, it could be predicted that hypertensive responses to exercise lead to excessive endothelial damage, increased arterial stiffness and acceleration of end-organ damage which would lead to the development of hypertension.

Using telephone interviews in the weeks following a myocardial infarction, it was suggested that myocardial infarctions were more likely to proceed heavy bouts of physical activity compared to periods of little or no physical activity (Mittleman and Siscovick, 1996, Willich et al., 1993). Interviews with patients or close family members following a non-fatal or fatal subarachnoid haemorrhage have also

highlighted that this condition is more likely to happen two hours following moderate to heavy physical exertion compared to rest or light exercise (Anderson et al., 2003, Schievink et al., 1989). Older patients were at increased risk of subarachnoid haemorrhage following moderate to heavy physical exertion with a history of hypertension, treated or untreated (Anderson et al., 2003). The authors postulated that sharp rises in BP in these individuals during the physical activity may predispose to MIs and/or subarachnoid haemorrhage. An obvious limitation to telephone interviews and face-to-face interviews is recall bias from participants and therefore may have limited the findings from these studies. Nevertheless, these studies did indicate that the sharp BP responses to exercise in hypertension predispose them to adverse CV and cerebrovascular events. This is concerning as clinicians are increasingly advising their patients with hypertension to take part in more physical exercise to lower resting BP.

Available evidence suggests that individuals from the general population, free from anti-hypertensive medication, with an exaggerated BP response to exercise are at increased risk of left ventricular hypertrophy (Ren et al., 1985, Papademetriou et al., 1989, Mizuno et al., 2016b), myocardial infarction (Laukkanen et al., 2006, Kjeldsen et al., 1997, Mundal et al., 1996, Kjeldsen et al., 2001, Filipovsky et al., 1992, Kohl et al., 1996), any type of stroke (Laukkanen et al., 2006, Kurl et al., 2001) and total mortality (Filipovsky et al., 1992, Fagard et al., 1996, Kohl et al., 1996) independent of resting BPs. These findings have been confirmed using both submaximal (Mundal et al., 1996, Filipovsky et al., 1992, Kjeldsen et al., 1997, Tzemos et al., 2015) and maximal exercise (Kurl et al., 2001, Laukkanen et al., 2006, Fagard et al., 1996, Ren et al., 1985, Kohl et

al., 1996). A recent meta-analysis of 46,314 individuals from the general population, including normotensives and untreated hypertensives concluded that an excessive rise in SBP during submaximal, but not maximal exercise were predictive of CV outcomes (Schultz et al., 2013b). The authors stated that the pooled hazard ratio for the studies included was 1.36 for submaximal exercise and 1.49 for maximal exercise SBP, which could indicate a trend towards biological significance (Schultz et al., 2013b). For every 10 mmHg increases in SBP during submaximal exercise, there was a 10% increase in the risk of an adverse CV event (Schultz et al., 2013b). Additionally, a low number of studies that have assessed the significance of maximal exercise SBP limited the statistical power for this analysis. It is interesting to note that the most consistent findings regarding the BP response to submaximal and maximal exercise have come from studies using cycle ergometer testing (Kurl et al., 2001, Laukkanen et al., 2006, Mundal et al., 1996, Filipovsky et al., 1992, Hietanen et al., 2010, Fagard et al., 1996, Kjeldsen et al., 2001), compared to studies using treadmill testing (Lewis et al., 2008, Ren et al., 1985, Weiss et al., 2010, Kohl et al., 1996, Shalnova et al., 1996). Measuring BP during treadmill testing is likely to be influenced by movement artefact more than during cycle ergometer testing and therefore BPs may not be as accurate (Laukkanen et al., 2006).

There is a lack of research assessing whether having hypertension and an exaggerated BP response to exercise places these individuals at increased CV and/or cerebrovascular risk compared to normotension or even compared to hypertensives with a more normal rise in BP. In addition, a large proportion of the studies assessing the prognostic value of exaggerated SBPs have relied on clinic

BP assessment to assess baseline BPs. (Franklin et al., 2013, O'Brien et al., 2013). Future studies will need to include 24-hour ABPM, and then participants will be properly defined as normotensive or hypertensive. The conflicting data that has arisen between cycle ergometer and treadmill exercise testing for the prognostic significance of moderate and maximal exercise BPs has led to the American college of cardiology/American Heart association (Gibbons et al., 2002) and the European Society of Hypertension/European Society of Cardiology (Mancia et al., 2007, Mancia et al., 2013) to include exaggerated BP responses to exercise being linked to future risk of hypertension but not CV disease, stroke or total mortality within their guidelines. Although more research in the area is needed, it does appear that an exaggerated SBP response to exercise does carry extra prognostic value independent of baseline BP values when measured during cycle ergometer exercise at both moderate and at maximal exercise. A large amount of evidence suggests that the autonomic response to exercise is exaggerated in patients with untreated hypertension. The research suggests that the exercise pressor reflex appears to be a key mediator of this exaggerated autonomic response to exercise in this disease.

1.6 The pathophysiology of exercise hypertension

1.6.1 The exercise pressor reflex in hypertension

1.6.1.1 Animal models

Both components of the exercise pressor reflex, the mechanoreflex and the metaboreflex are over-active in animal models of hypertension (Leal et al., 2008). In decerebrated SHR, activation of the metaboreflex via injections of capsaicin

directly into the hindlimb, which act on TPR_v1 receptors, lead to exaggerated changes in BP and RSNA when compared to Wistar-Kyoto rats (WKY) (Leal et al., 2008, Mizuno et al., 2011b, Mizuno et al., 2011a, Mizuno et al., 2015b). Similar findings of metaboreflex oversensitivity were found when using aldosterone and salt-loaded hypertensive rats (Mizuno et al., 2013, Mizuno et al., 2014b, Mizuno et al., 2015a). Importantly the BP response to acute treadmill exercise testing is also exaggerated in SHR compared to WKY, which would suggest exaggerated metaboreflex activation is related to impaired dynamic BP control (Kashimura and Igawa, 1996). In contrast, in canines made hypertensive by a reduction in renal blood flow via unilateral partial occlusion of the left kidney, activation of the metaboreflex via reductions in hindlimb blood flow during submaximal treadmill running following the induction of hypertension led to attenuated changes in BP, HR and CO (Spranger et al., 2017, Sala-Mercado et al., 2013). Several interpretations are possible 1) species variations in the metaboreflex 2) isometric vs dynamic exercise 3) decerebration of the rats vs conscious dogs and 4) the mechanisms mediating the rise in BP in order to develop hypertension. In the decerebrated rats the BP response to metaboreflex activation was primarily mediated by increased renal vasculature resistance (Smith et al., 2010, Smith et al., 2006, Leal et al., 2008). In contrast, in dogs the rise in BP during metaboreflex activation is primarily due to changes in CO (Sala-Mercado et al., 2013, Spranger et al., 2017). Following the induction of hypertension in the dogs, peripheral vasoconstriction increased but the attenuation of the CO caused reductions in BP during metaboreflex activation (Sala-Mercado et al., 2013, Spranger et al., 2017). The contribution of CO compared to peripheral vasoconstriction to the rise in BP during metaboreflex

activation is also likely mediated by isometric vs. dynamic exercise and also whether the metaboreflex is activated during or following exercise (Crisafulli et al., 2011). It was later shown that the impaired CO during metaboreflex activation in the dogs was due to sympathetic vasoconstriction of the coronary vasculature, which led to decrements in left ventricular performance (Spranger et al., 2017).

The mechanically sensitive afferents in decerebrated SHR using 30 seconds of passive stretching of the hindlimb also lead to exaggerated changes in BP, HR and RSNA when compared to WKY (Leal et al., 2008, Leal et al., 2013, Mizuno et al., 2011b).

Importantly, these results in SHR for both the metaboreflex and mechanoreflex remain the same after decerebration, which removes the influences of central command, and also following baro-denervation, which removes the influences of the baroreflex, highlighting the importance of the exercise pressor reflex in the SHR model of hypertension (Smith et al., 2010, Leal et al., 2008, Mizuno et al., 2011b). The exercise pressor reflex appears critical in mediating the abnormal sympathetic nervous system activity and BP response to exercise seen in animal models of hypertension.

1.6.1.1.1 Mechanisms for metaboreflex oversensitivity in animal models of hypertension

The exact mechanism driving the dysfunctional metaboreflex in animal models of hypertension is still unclear. Alterations in the metaboreflex could occur at any point in the reflex arc. Capsazepine, which antagonises the TRP_V1 receptors, reduced the MAP and RSNA response to capsaicin and ischaemic muscle contraction in SHR more than in WKY (Mizuno et al., 2011a). Interestingly, Western blot analysis revealed the density of the TRP_V1 receptors was elevated in SHR only in the dorsal root ganglion, but not in the skeletal muscle where the afferent nerve endings lie (Mizuno et al., 2011a). The authors speculated that enhanced phosphorylation of the TRP_V1 receptors in the skeletal muscle could lead to increased metaboreflex activity in SHR. Local administration of a non-specific antagonist of the purinergic receptors (P_2) receptors (pyridoxial-5-phosphate (PLP)) in to the antecubital vein, had a moderate effect on the BP and MSNA responses to isometric handgrip exercise and PEI in patients with untreated hypertension (Greaney et al., 2014). This finding highlights the documented synergy of the metaboreflex where all the metabolites together are more effective than one or two (Light et al., 2008, Jankowski et al., 2013). However, it also demonstrates the redundancy of the metaboreflex, by abolishing one of the metabolites that effects the metaboreflex, it will still remain sensitised during exercise in hypertension due to the presence of the other metabolites (Joyner, 2013, Stone et al., 2015). One of the issues with targeting the skeletal muscle receptors for the treatment of metaboreflex hyperreflexia is that we still do not fully understand the exact metabolites that increase its sensitivity in hypertension. Interestingly, a study in mice found that μ-opioid receptor

expression is higher on group IV afferents, whereas δ-opioid receptor expression is increased on group III afferents (Scherrer et al., 2009). Therefore, the use of fentanyl, which is an agonist for μ-opioid receptor may highlight alterations at the spinal cord in the processing of the metaboreflex (Barbosa et al., 2016).

Alterations in central processing of afferent signals from the skeletal muscle may also contribute to an exaggerated metaboreflex sensitivity. The same level of electrical stimulation to activate the afferent nerve fibres in decerebrated SHR and WKY led to exaggerated BP increases in the SHR (Smith et al., 2006). This suggested that the same level of group III and IV afferent information is interpreted differently within the dorsal horn and/or within the brain stem in hypertension. Increasing central NO bioavailability within the NTS decreases the sympathetically mediated changes in MAP associated with activation of the exercise pressor reflex (Smith et al., 2005a). In SHR there are fewer neurons that express nNOS when compared to WKY in areas of the NTS that are excited by the group III and IV afferents (Murphy et al., 2013). Dialysis of L-arginine in the NTS reduced the exaggerated increase in BP in decerebrated SHR to levels seen in WKY (Leal et al., 2013). Similarly, inhibiting the endogenous production of NO via dialysis of L-NAME into the NTS led to exaggerated BP responses to contraction in WKY which were similar to SHR (Leal et al., 2012). In addition, angiotensin II production is exaggerated in individuals with an exaggerated BP response to exercise (Shim et al., 2008, Williams et al., 2013). A recent study suggested that, in healthy people, a centrally acting ACE inhibitor (perindopril) was more effective at lowering MSNA and BP during exercise than a peripherally acting ACE inhibitor (captopril) (Moralez et al., 2018). Centrally elevated levels of

angiotensin II may reduce the capacity of the NTS to buffer the elevated afferent activity of the group III and IV afferents in hypertension, leading to excessive efferent MSNA.

A recent study found that 3-weeks of spironolactone or eplerenone, both mineralocorticoid receptor antagonists, attenuated the exaggerated BP response to electrical stimulation of the hindlimb muscles in SHR but had no effect on WKY (Downey et al., 2017). Unfortunately, the oral administration of spironolactone or eplerenone limited the group's ability to identify which part of the reflex arc the antagonists were blocking. Research has shown that there are high levels of mineralocorticoid receptors at the level of the dorsal horn (González et al., 1992) and within the NTS (Sequeira et al., 2006). Future studies will need to confirm the targets of mineralocorticoid receptor antagonists and further the research to exercise pressor reflex in human hypertension.

1.6.1.2 Human hypertension

In older human hypertensives (mean age > 60 years) withdrawn from their anti-hypertensive medications 48-hours prior to participation, isolation of the metabolic component of the exercise pressor reflex using PEI leads to exaggerated increases in both MSNA (Delaney et al., 2010, Greaney et al., 2014) and SBP (Sausen et al., 2009, Delaney et al., 2010, Greaney et al., 2014) when compared to age-matched normotensive individuals. Metaboreflex sensitivity is also increased in younger never-treated prehypertensive individuals (mean age: 35±3 years old) compared to age-matched normotensives (mean age: 33±3

years old) (Choi et al., 2013). To assess whether generalised sympathoexcitability was the cause of the differences between hypertension and normotension in these studies, several used a cold pressor test (Sausen et al., 2009, Delaney et al., 2010, Choi et al., 2013) and in response to the cold pressor test, MSNA and BP were similar in both groups (Choi et al., 2013, Delaney et al., 2010, Sausen et al., 2009). In contrast, it was found that BP and MSNA were blunted during PEI in middle-aged never-treated hypertensive individuals (mean age: 42±1 years old) (Rondon et al., 2006). Although the disparity in results remains unclear, it could be due to the method chosen for assessment of BP (beat-to-beat assessment using photoplethysmography (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014, Choi et al., 2013) vs. BP measurements once per minute from the ankle used by Rondon et al. (2006).

Owing to difficulties in assessing the mechanical component of the exercise pressor reflex, very little research has assessed the mechanoreflex in humans with hypertension. Two different methods of mechanoreflex activation have been assessed. Firstly, based on Kaufman et al. (1983) finding that the mechanoreceptors fire rapidly at the onset of isometrically induced contraction in cats, the SBP and MSNA responses to the first 10-30s seconds of isometric handgrip exercise have been assessed in untreated hypertension (Greaney et al., 2015a). They found that even within 10s of isometric handgrip exercise at 30 and 40% maximal voluntary contraction (MVC) that SBP and MSNA were elevated in older untreated individuals with hypertension (mean age: 62±1 years old), withdrawn from their anti-hypertensive medications 48-hours prior to investigation, when compared to age-matched normotensive controls (mean age:

60 ±1 years old) (Greaney et al., 2015a). This result was taken to suggest increased mechanoreflex sensitivity in human hypertension. However, it is known that at the onset of isometric handgrip the autonomic response is also mediated by central command (Goodwin et al., 1972). Therefore, it is very difficult to draw specific conclusions regarding the mechanoreflex from this study. A more specific method to assess mechanoreflex sensitivity in humans is passive cycling using motorised pedals (Williamson et al., 1995). Passive arm cycling in untreated hypertension leads to exaggerated MSNA and BP when compared to age-matched normotensives (Velasco et al., 2015). It is clear that both the metaboreflex and mechanoreflex sensitivity both appear to be elevated in patients with untreated hypertension when compared to age-matched individuals.

In patients with untreated hypertension no differences in the control of HR are seen during PEI following isometric and dynamic handgrip exercise (Rondon et al., 2006, Sausen et al., 2009, Delaney et al., 2010, Choi et al., 2013, Greaney et al., 2014). During PEI following arm exercise, sympathetically mediated increases in HR are buffered by a synchronous increase in cardiac parasympathetic tone (Fisher et al., 2013, Fisher et al., 2010). This increased parasympathetic tone occurs due to withdrawal of mechanoreflex activity (Gladwell et al., 2005) and central command (Krogh and Lindhard, 1917, Goodwin et al., 1972). However, following exercise using a large muscle mass (e.g dynamic cycle ergometer), HR is increased or maintained during PEI (e.g., cycle ergometer) which invokes a larger metaboreflex mediated increase in SNA and withdrawal of vagal tone that is maintained during PEI (Fisher et al., 2013). Whether differences in HR would be found between hypertension and normotension during PEI following cycle

ergometer testing remains to be seen. On the contrary, the mechanoreflex appears to mediate exaggerated increases in HR in untreated hypertension relative to age matched normotensives (Greaney et al., 2015a, Velasco et al., 2015). Lumbar intrathecal fentanyl (μ-opioid receptor agonist) normalised the SBP response to low intensity (40 watts) cycle ergometer exercise in untreated hypertensive (mean age: 49±2 years old) individuals relative to normotensives (mean age: 47±5 years old), highlighting a critical role for the exercise pressor reflex in untreated hypertension (Barbosa et al., 2016). Unlike the other studies in hypertension, this study classified untreated hypertension and normotensives using 24-hour ABPM.

A final possible mechanism mediating enhanced metaboreflex sensitivity in hypertension is impaired functional sympatholysis. This would lead to a supra-physiological rise in metabolites that activate the metaboreflex and lead to an exaggerated sympathetic and BP response during exercise (Mizuno et al., 2014a, Choi et al., 2013, Saltin and Mortensen, 2012).

1.6.1.3 Functional sympatholysis in hypertension: insights from animal models

Research in hypertensive animals assessing functional sympatholysis has shown that rats made hypertensive by angiotensin II infusion (Zhao et al., 2006), SHR (Mizuno et al., 2014a) and dogs with renovascular hypertension (Sala-Mercado et al., 2013, Spranger et al., 2017) have impaired functional sympatholysis compared to normotensive animals. The nNOS splice variant sarcolemmal nNOSμ is particularly abundant in skeletal muscle, and in combination with eNOS

in the blood vessels, enables matching of O_2 supply and demand (Thomas et al., 1998, Thomas et al., 2003). In mice, NO produced from nNOSμ has been shown to be able to diffuse from local skeletal sarcolemma in to resistance vessel vascular smooth muscle where it attenuates post-junctional α_1- and α_2-adrenoceptors (Thomas et al., 1998, Thomas et al., 2003). During exercise, skeletal muscle NO levels are diminished in rats made hypertensive by infusion of angiotensin II compared to control animals (Zhao et al., 2006). Similar to the brainstem, decreased NO bioavailability is partially mediated by increased levels of angiotensin II, noradrenaline and ROS (Zhao et al., 2006). The levels of NAD(P)H and O_2^- were elevated during exercise in the active skeletal muscle in anaesthetized hypertensive rats compared to control anaesthetized animals (Minuz et al., 2002, Zhao et al., 2006). Importantly, increased levels of O_2^- in hypertensive rats were normalised when the rats were given tempol and functional sympatholysis was restored (Zhao et al., 2006). However, when these animals received the non-specific NOS inhibitor L-NAME, the normalisation of functional sympatholysis was reversed, highlighting a key role for NO in hypertensive rats (Zhao et al., 2006). Additionally, functional sympatholysis is normalised in endurance trained SHR relative to WKY (Mizuno et al., 2014a). When the endurance trained SHR were given L-NAME, the improvements in functional sympatholysis were diminished (Mizuno et al., 2014a). Suggesting a key role of NO in mediating improvements in functional sympatholysis seen with endurance training. Recent studies have attempted to move these studies from animals with experimentally induced hypertension in to humans with essential hypertension.

1.6.1.3.1 Humans

In humans, functional sympatholysis is assessed using vascular ultrasound (to measure forearm/femoral blood flow) and forearm muscle oxygenation levels using near infrared spectroscopy (NIRS). Firstly, forearm blood flow and forearm vascular conductance increased similarly in middle aged patients with untreated essential hypertension and normotensives during dynamic handgrip exercise at 30% MVC for 6 minutes (Vongpatanasin et al., 2011). At rest, lower body negative pressure at -20 mmHg induced similar decreases in forearm blood flow, forearm vascular conductance and forearm muscle oxygenation levels in untreated hypertensives and normotensives (Vongpatanasin et al., 2011). Interestingly, there was an attenuated decrease (compared to rest) in forearm blood flow, forearm vascular conductance and muscle oxygenation levels when an additional sympathetic stimulus (lower body negative pressure at -20 mmHg) was applied during minutes 3-5 of the dynamic handgrip exercise at 30% MVC in normotensive participants, indicating functional sympatholysis (Vongpatanasin et al., 2011). However, in the untreated hypertensive patients, forearm blood flow, forearm vascular conductance and muscle oxygenation levels were reduced to a similar level as rest when lower body negative pressure at -20 mmHg was applied during minutes 3-5 of the dynamic handgrip exercise, indicating impaired functional sympatholysis (Vongpatanasin et al., 2011). In contrast, infusion of adenosine (which stimulates the formation of NO and prostacyclin) into the femoral artery of untreated hypertensive and normotensive participants led to a similar increase in leg blood flow and vascular conductance (Hellsten et al., 2012). However, exercise induced changes in interstitial adenosine were reduced in untreated hypertensives compared to normotensives (Hellsten et al., 2012).

Furthermore, during one-legged knee extensor exercise at 20 watts, femoral blood flow and femoral vascular conductance were lower in untreated hypertensives compared to normotensives (Hellsten et al., 2012). Similar findings were found when measuring femoral blood flow and femoral vascular conductance in the last 30 seconds of one-legged knee extensor exercise (30 watts) in patients with untreated hypertension (Mortensen et al., 2014). However, tyramine infusion into the femoral artery during one-legged knee extensor exercise (30 watts) caused a similar reduction in femoral blood flow and vascular conductance in untreated hypertensives and normotensives (Mortensen et al., 2014). Leg $\dot{V}O_2$ was reduced in untreated hypertensives compared to normotensives with tyramine infusion during one-legged knee extensor exercise at 30 watts which may suggest impaired perfusion during leg exercise (Mortensen et al., 2014). Interestingly, previous literature has highlighted differences in vascular function between the arms and the legs (Pawelczyk and Levine, 2002). For example, infusion of phenylephrine into the brachial and femoral artery led to greater reductions in vascular conductance in the calf compared to the forearm in healthy individuals (Pawelczyk and Levine, 2002). Furthermore, due to the inconsistencies in the literature more research is needed to assess differences in functional sympatholysis in the leg and arms in untreated hypertension when compared to normotension.

1.6.2 Link between metaboreflex over-sensitivity and impaired functional sympatholysis

In patients with untreated hypertension, impaired functional sympatholysis augments a mismatch between O_2 supply and demand, which facilitates an increase in metabolites that sensitise the metaboreflex. An exaggerated increase in SNA, mediated by the metaboreflex would likely lead to further vasoconstriction, consequently stimulating the metaboreflex further in a viscous cycle (Figure 1.5, page 89). In untreated hypertension, it could be speculated that as exercise intensity increases, CO rises alongside an excessive rise in TPR. This leads to abnormally large increases in BP. A recent study suggested that increased metaboreflex sensitivity in prehypertension is associated with exaggerated increases in TPR (Choi et al., 2013). Additionally, functional sympatholysis was improved in patients with untreated hypertension with irbesartan (Vongpatanasin et al., 2011) and nebivolol (Price et al., 2013) and both were associated with a lower BP response to exercise, but not improvements in MSNA when compared to pre-treatment measurements. No studies have directly established whether increases in metaboreflex sensitivity are associated with impaired functional sympatholysis in hypertension. However, some indirect evidence comes from peripheral artery disease (PAD), which is characterised by impaired perfusion during exercise (Bakke et al., 2007). An animal model of PAD in rats is initiated by ligating the femoral artery that simulates the impaired functional sympatholysis during exercise but maintains normal blood flow to resting skeletal muscle (Stone and Kaufman, 2015). Femoral artery ligation in rats leads to increased expression of ASIC3 (Liu et al., 2010, Xing et al., 2012), $P_{2X}3$ (Xing et al., 2013), bradykinin B2 (Lu et al., 2013), endoperoxide 4

(Yamauchi et al., 2013) receptor proteins on the peripheral endings of the group IV afferents. Importantly, these changes lead to exaggerated RSNA and BP during static contraction when compared to freely perfused rats (Liu et al., 2010, Xing et al., 2012, Xing et al., 2013, Lu et al., 2013, Yamauchi et al., 2013). Although these studies were not conducted in hypertension, blood flow, vascular conductance and $\dot{V}O_2$ in the active skeletal muscle have been shown to be impaired in untreated hypertension, which would suggest under-perfusion to the active skeletal muscle (Mortensen et al., 2014, Vongpatanasin et al., 2011). In addition, Mortensen et al. (2014) found that resting blood flow and vascular conductance were also impaired in the resting skeletal muscle. It could be speculated that impaired functional sympatholysis during exercise in hypertension leads to an increased expression of receptors that activate the metaboreflex. Metaboreflex hyperreflexia would lead to exaggerated increases in SNA, which would cause further vasoconstriction and perhaps further increase the expression of the receptors.

1.6.3 Why focus on the metaboreflex?

Although both the mechanoreflex and metaboreflex appear critical in mediating the abnormal CV response to exercise in hypertension, in this thesis I have focused on the metaboreflex. Firstly, assessing the metaboreflex in humans using PEI is much easier to assess independent of central command and mechanoreflex activity when compared to measuring the mechanoreflex sensitivity via passive cycling (Velasco et al., 2015), immediate CV responses to isometric handgrip exercise (Greaney et al., 2015a) and passive stretch (Choi et

al., 2013). After several attempts to perform passive cycling to isolate the mechanoreflex I stopped as without electromyography (EMG) to confirm the absence of active muscle movement I could not be confident of mechanoreflex isolation. Additionally, more is known about the mechanisms that mediate the metaboreflex in human hypertension and pharmacological options exist that could be used to dampen down its sensitivity. Little is known about the specific mechano-gated receptors that mediate the mechanoreflex component of the exercise pressor reflex in human hypertension. Therefore, at this moment, potential treatment for mechanoreflex oversensitivity in human hypertension is very limited.

1.6.4 How do antihypertensive medications influence the BP response to exercise?

There is a lack of studies investigating the effects of anti-hypertensive medications on the BP response to exercise. This is important to assess because of the relationship between exaggerated BP responses to exercise and CV events. The long term haemodynamic effects of anti-hypertensive medications on *mild* intensity cycling exercise at 100 watts in middle aged patients with hypertension were assessed by Omvik and Lund-Johansen (1993), they found similar reductions in BP at rest and during exercise regardless of drug class. A different study found that only β-adrenoceptor antagonists, long-acting calcium channel blockers and ACEi were effective at lowering exercise BP at submaximal cycling exercise (Arita et al., 2001) compared to α_1-adrenoceptor antagonists, thiazide like diuretics or short-acting calcium channel blockers. Alternatively,

other studies have found that only chronic use of β-adrenoceptor antagonists are effective at lowering BP during moderate and maximal treadmill exercise compared to an ACEi, calcium channel blocker or diuretic (Kokkinos et al., 2006). All of these studies lacked healthy controls, so we do not know if antihypertensive medications lower BP to normal levels during exercise. The inconsistency from these studies could be due to differences in drugs, such as half-life and exercise pharmacokinetics, different exercise intensities (submaximal vs. maximal) and exercise modes used (treadmill vs. cycling exercise). One study assessed the prognostic value of exaggerated BP response to exercise in 300 patients with hypertension withdrawn from their anti-hypertensive medication or who were untreated, confirmed by 24-hour ABPM, free from CV disease (except hypertension) (Cho et al., 2012). 87.1% of these individuals were taking anti-hypertensive medication prior to withdrawal and these individuals had their BP response to a submaximal Naughton/Balke treadmill test measured. The individuals with the largest BP response to exercise had an increased risk of mortality, ischemic heart disease and stroke, independent of resting BP (Cho et al., 2012). An exaggerated BP response to exercise following 12 months of anti-hypertensive was associated with depressed anti-hypertensive treatment-induced regression of left ventricular hypertrophy (Mizuno et al., 2016b). Unfortunately, this study lacked a control group of normotensives. Additionally, the study assessing left ventricular hypertrophy did not follow up individuals long term to see if these individuals were at increased risk of CV disease and/or mortality (Mizuno et al., 2016b).

These studies have left two important questions unanswered:

1) Does adequately controlling BP based on 24-hour ABPM normalise the BP response to exercise?
2) If adequate control of resting BP doesn't normalise the BP response to exercise, is this group at increased risk of adverse events compared to normotensive controls? The **main aim** of this thesis is to answer question one.

We currently do not know if anti-hypertensive medication that normalises baseline BP restores metaboreflex sensitivity to normotensive levels (Figure 1.5, page 89). The current anti-hypertensive treatments do not act on the known receptors that activate the skeletal muscle metaboreflex (Figure 1.5, page 89). Two important pathways have been targeted in human hypertension for the treatment of insufficient functional sympatholysis, ATP (Mortensen et al., 2014) and NO (Price et al., 2013, Vongpatanasin et al., 2011). Interestingly, anti-hypertensives that have similar effects on lowering BP at rest have differential effects on functional sympatholysis (Price et al., 2013, Vongpatanasin et al., 2011). Four weeks of ibersartan, an AT_1 receptor antagonist, or a thiazide-like diuretic (chlortalidone), caused similar reductions in resting BP but only the AT_1 receptor antagonist caused improvements in functional sympatholysis (Vongpatanasin et al., 2011). This supports a key role of angiotensin II in mediating excessive vasoconstriction in the active skeletal muscle (Zhao et al., 2006, Rajagopalan et al., 1996). In a follow-up to this study it was shown that 12 weeks of nebivolol, a selective β-adrenoceptor 1 antagonist with both antioxidant and NO potentiating properties, improved functional sympatholysis during

handgrip in patients with untreated hypertension compared to a conventional β_1 adrenergic antagonist (metoprolol) (Price et al., 2013).

Interestingly, in both of these studies, the anti-hypertensive drugs had no effect on the level of SNA during handgrip or handgrip with lower body negative pressure (Price et al., 2013, Vongpatanasin et al., 2011). The antioxidant properties of nebivolol may have caused a reduction in ROS decreasing O_2^- production, thereby increasing the bioavailability of NO (Zhao et al., 2006). The vasodilatory effect of nebivolol in the renal vasculature is mediated by an increased P_{2Y} receptor activation via augmented ATP efflux from the endothelial cells, which leads to a calcium-dependent activation of eNOS (Kalinowski et al., 2003). Although this has not been assessed in skeletal muscle, it does suggest a possible interaction between ATP and NO in the human skeletal muscle during exercise. However, Mortensen et al. (2014) found no difference in the vasodilatory response to femoral arterial ATP infusion in patients with untreated hypertension compared to healthy controls.

Further work is needed to establish the mechanism underlying impaired functional sympatholysis in human hypertension. Therefore, increased sensitivity of the metaboreflex in treated-controlled hypertension could lead to exaggerated rises in MSNA that will not be correctly offset by functional sympatholysis during exercise which would be expected to lead to an elevated BP response and place these individuals at increased CV risk.

Increasing NO bioavailability in patients with hypertension may therefore be a therapeutic avenue to explore to decrease metaboreflex hyperreflexia during exercise. However, the replacement of substrates and co-factors (such as L-arginine) in hypertension have not been successful in improving vascular function (Boger, 2007, Schulman et al., 2006, Wilson et al., 2007), perhaps because they rely on a healthy endothelium. Furthermore, NO donors such as nitroglycerin lead to endothelial dysfunction (Fadel et al., 2012) and are prone to tachyphylaxis (Fadel et al., 2012). Nebivolol may improve functional sympatholysis during rhythmic handgrip exercise in untreated hypertension (Price et al., 2013), but β-adrenoceptor antagonists are known to impair the CV response to exercise at submaximal and maximal intensities (Van Baak, 1988). Therefore, therapeutics could be investigated that aim to improve metaboreflex hyperreflexia and functional sympatholysis (but do not limit the CV response to exercise).

1.7 Dietary Nitrates

It was originally thought that NO was generated solely by the oxidation of L-arginine, which results in the oxidation of NO to nitrite (NO_2^-) and nitrate (NO_3^-) (Moncada and Higgs, 1993). NO_3^- and NO_2^- were thought to be inert end products of the oxidation of NO (Mensinga et al., 2003). However, since 2001 it has been discovered that dietary sources of inorganic NO_3^- can be reduced to NO_2^- and NO in various tissues and represent an alternative NO pathway (NO_3^--NO_2^- - NO pathway) (Webb et al., 2008b, Demoncheaux et al., 2002). The NO_3^--NO_2^- - NO pathway appears to complement the classical L-arginine pathway (Kapil et al., 2010). The greatest source of dietary inorganic NO_3^- comes from

leafy green vegetables such as beetroot, rocket and spinach which contain > 250 mg (> 4mmol) of dietary NO_3^- per 100 g. To put this into context, the typical intake of dietary NO_3^- in the non-vegetarian United Kingdom population is around 90 mg/day (Ysart et al., 1999).

In humans, upon swallowing dietary NO_3^-, ~25% enters the enterosalivary circulation where NO_3^- is concentrated within the salivary glands (Duncan et al., 1995). It is at this site where NO_3^- is reduced to NO_2^- by a 2-electron reduction by symbiotic bacterial NO_3^- reductases on the lingual portion of the tongue (Duncan et al., 1995). Using gene sequencing techniques, the specific species that reduce NO_3^- have been identified as the *Veillonella, Prevotella, Neisseria, Haemophilus* and *Actinomyces* (Hyde et al., 2014). The importance the oral microflora was demonstrated by studies using antibacterial mouthwash, which effectively diminished the normal rise in plasma NO_2^- following NO_3^- ingestion (Woessner et al., 2016, Kapil et al., 2013). A portion of the swallowed NO_2^- is reduced to NO due to the acidity within the stomach, however, NO_2^- also enters the circulation and leads to a substantial rise in plasma NO_2^- levels (Webb et al., 2008b). Following ingestion, plasma NO_3^- levels rise sharply (within ~30 minutes), peak at around 2-3 hours and remain elevated when compared to basal levels for 24-hours post-ingestion (Kapil et al., 2010). Plasma NO_2^- levels rise slowly and are noticeable within 1.5 - 2 hours following ingestion and plateau at around 2.5 hours, similar to NO_3^-, NO_2^- levels remain elevated 24-hours following ingestion (Kapil et al., 2010). Importantly, the one electron reduction of NO_2^- to NO occurs particularly when the partial pressure of O_2 is reduced or in conditions of low pH

(Modin et al., 2001), conditions that are likely to occur in the skeletal muscle during exercise (Richardson et al., 1995).

The reduction of NO_2^- to NO is endothelial independent and is carried out in the plasma and in various bodily tissues by a variety of NO_2^- reductases including deoxygenated haemoglobin (see Figure 1.4, page 87) (Cosby et al., 2003, Gladwin and Kim-Shapiro, 2008), deoxygenated myoglobin (Shiva et al., 2007), neuroglobin (Tiso et al., 2011, Petersen et al., 2008), xanthine oxidoreductase (Ghosh et al., 2013, Webb et al., 2004, Webb et al., 2008a, Li et al., 2008, Badejo et al., 2010), aldehyde oxidase (Li et al., 2008), the electron transport chain (Kozlov et al., 1999), cytochrome p450 reductase (Li et al., 2006), mitochondrial aldehyde dehydrogenase (Badejo et al., 2010) and carbonic anhydrase (Aamand et al., 2009). Under physiological conditions, where partial pressure of O_2 and pH are not extremely low, the main mechanism mediating NO_2- to NO is deoxygenated haemoglobin (Cosby et al., 2003) (see Figure 1.4, page 87). Importantly there is a temporal pattern of increased plasma nitrite and cGMP levels, indicating that plasma nitrites lead to increased NO activity (Kapil et al., 2010). Additionally, unlike other NO donors (e.g., nitroglycerin), dietary NO_3^- do not suffer from tachyphylaxis (Kapil et al., 2015, Vanhatalo et al., 2010).

Short term (2-6 days) dietary NO_3^- can reduce the O_2 cost ($\dot{V}O_2$) of exercise at a fixed power output (Larsen et al., 2007, Bailey et al., 2009), improve exercise efficiency (Larsen et al., 2007, Bailey et al., 2009), increase time to exhaustion (Bailey et al., 2010, Larsen et al., 2010, Thompson et al., 2014) and improve

'real-world' performance (Lansley et al., 2011a, Wilkerson et al., 2012) in healthy athletic populations. These effects are maintained following 15 days of dietary NO_3^- supplementation (Vanhatalo et al., 2010) and importantly when compared to a placebo (NO_3^--depleted) (Lansley et al., 2011b, Lansley et al., 2011a). Physiologically, these changes in exercise performance are in part mediated by improvements in mitochondrial respiration (decreases in ATP turnover) (Bailey et al., 2010), oxidative phosphorylation (Larsen et al., 2011) and the contractile function of fast twitch skeletal muscle fibres (Hernandez et al., 2012).

Interest in dietary NO_3^- for CV protection has increased in recent years, partly because leafy green vegetables confer the largest benefit to CV health compared to other vegetables (Joshipura et al., 2001). The Japanese diet, which is traditionally very high in dietary NO_3^- is associated with lower resting SBP and DBP when compared to the Western diet in the Japanese population (Sobko et al., 2010, Sadakane et al., 2008). Acute (Kapil et al., 2010, Bondonno et al., 2012, Liu et al., 2013, Bahra et al., 2012, Lansley et al., 2011a, Webb et al., 2008b) and chronic (Larsen et al., 2007, Bailey et al., 2009, Ashworth et al., 2015, Jovanovski et al., 2015, Lansley et al., 2011a, Bailey et al., 2010, Vanhatalo et al., 2010) use of dietary NO_3^- has been shown to lower resting clinic SBP and DBP in healthy individuals in a dose-dependent manner, with at least a dose of 2-3 mmol/day of dietary NO_3^- (~200 g of spinach) needed to confer this CV benefit (Hobbs et al., 2012, Bondonno et al., 2012, Sobko et al., 2010). Doses of up to 24 mmol have been shown to lower BP in healthy individuals, however, this dosage may be very hard to achieve through a normal diet (Kapil et al., 2010). These finds have also been replicated when using 24-hour ABPM in

healthy volunteers following acute dietary NO_3^- intervention but not chronic interventions (Hobbs et al., 2012, Coles and Clifton, 2012).

Importantly, in SHR, there is a dose-dependent decrease in resting BP from NO_2^-, that is not apparent in Wystar-Kyoto rats (Beier et al., 1995, Classen et al., 1990, Haas et al., 1999). Similar findings have been found in salt-induced hypertension (Carlstrom et al., 2011). Mechanistically, expression of the NO_2^- reductase xanthine oxidoreductase was increased in the liver (a major site for xanthine oxidoreductase synthesis) and erythrocytes in SHR compared to the Wystar-Kyoto rats (Ghosh et al., 2013). Furthermore, this suggests that dietary NO_3^- may be more effective in hypertension than in those with normal BP. In the same study the BP lowering effect of dietary NO_3^- was blocked by allopurinol (a xanthine oxidoreductase antagonist) (Ghosh et al., 2013). Secondly, dietary NO_3^- decreased vascular NAD(P)H oxidative stress in two-kidney one clip hypertensive rats (Montenegro et al., 2011).

Dietary NO_3^- have also been shown to lower resting BP in humans with hypertension. Ghosh et al. (2013) showed that 3.3 mmol of dietary NO_3^- (beetroot juice) acutely decreased clinic SBP in treatment naïve males and females with hypertension. The same group then did a larger 4-week randomised, double-blinded, placebo-controlled, crossover (2-week run-in) study and found that treated middle-aged males and females with elevated resting BP had a reduction in clinic, 24-hour ABPM and home BP monitoring following 6.4 mmol/day dietary NO_3^- compared to the placebo (Figure 1.6, page 90). (Kapil et al., 2015). This

study also found that pulse wave velocity, augmentation index and flow mediated dilatation were improved in the dietary NO_3^- group (Kapil et al., 2015). Most importantly, unlike the endothelium, which is dysfunctional in hypertension, this study suggested that the entero-salivary circulation is intact and that a diversity of oral bacteria exist in patients with hypertension which is able to perform the critical reduction of NO_3^- to NO_2^-. In contrast, similar studies have found short term dietary NO_3^- for 1-week have no effect on ambulatory BP or home BP monitoring in treated (Bondonno et al., 2015) and pre (Bondonno et al., 2014) hypertensives compared to a placebo. Reasons for this discrepancy remain unclear, however, dietary NO_3^- may be more effective in individuals with higher BP at rest. For example, the BP lowering effect of anti-hypertensive medications is enhanced with increasing resting BP (Law et al., 2003). Additionally, a substantial drop in resting BP may not be expected in individuals who already have treated-controlled hypertension at rest (Bondonno et al., 2015). The final possibility is the length of intervention with dietary NO_3^- (Kapil et al., 2015). Kapil et al. (2015) used 4-weeks of treatment compared to the 1-week of dietary NO_3^- used by Bondonno et al. (2015). It may be possible that longer treatment is needed for dietary NO_3^- to be efficacious at lowering resting BP.

1.7.1 Dietary nitrates, functional sympatholysis and the metaboreflex

Very little is known about dietary NO_3^-, functional sympatholysis and the metaboreflex. Sprague-Dawley rats fed beetroot juice for 5 consecutive days had improved functional sympatholysis in blood vessels suppling fast-twitch muscle fibres (type IIb) and reduced exercising BP during treadmill exercise when

compared to NO_3^--free water fed rats (Ferguson et al., 2013). Similar results have been found in healthy humans; it was shown that a NO_2^- infusion reduced vasoconstrictor tone at rest and improved functional sympatholysis during rhythmic handgrip exercise and reduced MAP as compared to a saline (placebo) infusion (Cosby et al., 2003).

Acute ingestion of dietary NO_3^- (in the form of beetroot juice) has also recently been shown to improve forearm blood flow and forearm vascular conductance during rhythmic handgrip exercise at 15 and 25% of MVC when compared to a placebo in healthy young individuals (Richards et al., 2018). In pre-hypertensive males, a dietary NO_3^- intervention improves endothelial function (as measured by flow-mediated dilatation), improves functional sympatholysis and decreases SBP at rest and during cycle ergometer exercise (Choi et al., 2016). In addition to its effects on peripheral vasodilation, dietary NO_3^- and NO_2^- are also able to cross the brain-blood barrier and lead to improvements in brain perfusion (Presley et al., 2011), cerebrovascular resistance (Bond et al., 2013) and decrease central sympathetic outflow (Notay et al., 2017) at rest and during isometric plus dynamic exercise. Acute dietary NO_3^- supplementation was shown to decrease MSNA at rest and during isometric handgrip exercise in young healthy individuals compared to a placebo (Notay et al., 2017). NO is also known to act as an inhibitory neuronal messenger within the carotid body (Wang et al., 1994). The effect of dietary NO_3^- on the carotid body is unknown but increasing NO bioavailability may decrease the activity of the carotid body. Finally, a recent study found that metaboreflex sensitivity, assessed by the change in SBP was

reduced in older adults by four weeks of dietary NO_3^- supplementation compared to a placebo (Schneider et al., 2018).

Currently, whether dietary NO_3^- intervention will decrease the sensitivity of the metaboreflex and reduce exercise BP in hypertension is unknown. It could therefore be speculated that by improving functional sympatholysis, and therefore lowering the level of metabolites that sensitise the metaboreflex, the exaggerated SNA response seen during exercise will be attenuated in hypertension. This could explain why the BP response to exercise was attenuated during exercise following a dietary NO_3^- intervention in pre-hypertensive males (Choi et al., 2016). Additionally, as dietary NO_3^- and NO_2^- are able to cross the blood-brain-barrier they could also improve the central processing of the metaboreflex. It is currently unclear what effect dietary NO_3^- will have on the BP response to exercise in individuals with hypertension who are currently treated and controlled by anti-hypertensive medication.

The current literature suggests that the first-line treatment for hypertension has mixed effects on functional sympatholysis, it could be suggested that dietary NO_3^- could improve the underlying physiological abnormalities that contribute to an exaggerated BP response to exercise in individuals with hypertension as well as improving exercise performance. Most importantly, dietary NO_3^- could be a low-cost method for making exercise safer in the hypertensive population.

1.8 Aims and hypotheses

The **overall aim** of this thesis is to assess whether adequate control of BP with anti-hypertensive medication normalises the exaggerated pressor response to exercise that is associated with untreated hypertension. It is **hypothesised** that anti-hypertensive medication will have no effect on the CV response to exercise in hypertension and that treatments that reduce metaboreflex hyperreflexia in hypertension will prove more beneficial.

The **primary aim** of *Chapter three* of this thesis is to assess the difference in SBP response to dynamic exercise ($\dot{V}O_2$ peak test) and metaboreflex isolation (using post-exercise ischemia) between untreated hypertension, treated-uncontrolled hypertension and treated-controlled hypertension compared to normotensive controls**.** Based on previous research, the **hypothesis** was that there would be a difference in the BP response to incremental cycle ergometer exercise and metaboreflex isolation between normotensive controls versus treated-controlled, treated-uncontrolled and untreated patients with hypertension.

In *Chapter four*, the **primary aim** is to assess whether elevated arterial stiffness (carotid-femoral pulse wave velocity) and increased central aortic SBP (arterial tonometry) at rest are associated with an elevated SBP response to $\dot{V}O_2$ peak testing in untreated hypertension, treated-uncontrolled hypertension and treated-controlled hypertension compared to normotensive controls. **It is hypothesised** that elevated arterial stiffness and central aortic SBP at rest will be associated

with an exaggerated SBP response to $\dot{V}O_2$ peak testing in normotensives, treatment controlled, uncontrolled and untreated hypertensive individuals.

The **main aim** of *Chapter five* is to assess whether dietary NO_3^- supplementation for 4 weeks can reduce metaboreflex hyperreflexia and therefore the SBP response to $\dot{V}O_2$ peak testing in patients with treated-controlled hypertension compared to a placebo. **It is hypothesised** that there will be a change in the SBP response to $\dot{V}O_2$ peak testing and metaboreflex isolation following dietary NO_3^- supplementation for 4 weeks when compared to a placebo.

1.9 Figures

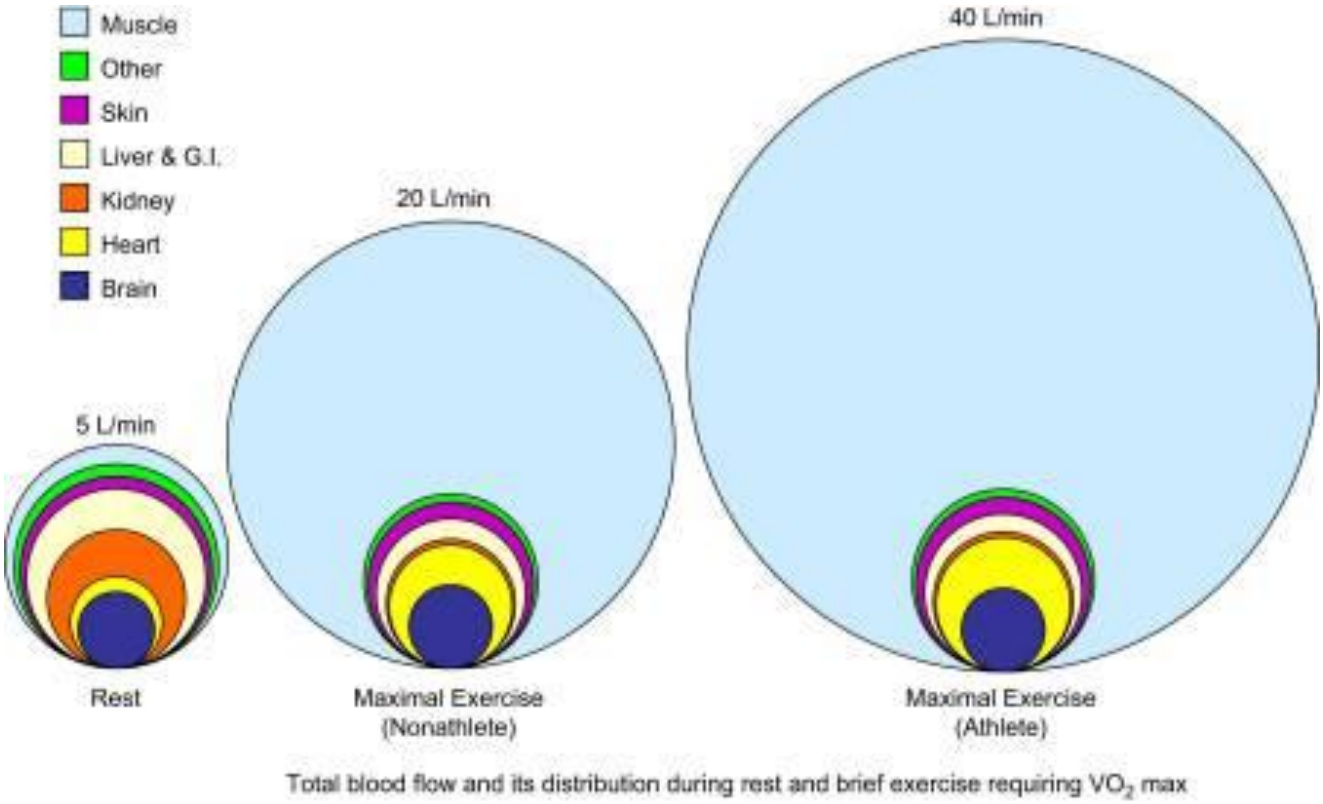

Figure 1-1 Total blood flow at rest and during exercise in a nonathlete and athlete. At rest, with a normal CO of 5 L/min the distribution of blood is relative to oxygen demand. As exercise intensity increases and CO and the demand for oxygen is increased in the skeletal muscle, skeletal muscle blood flow increases to match the oxygen demand [from Joyner and Casey (2015)].

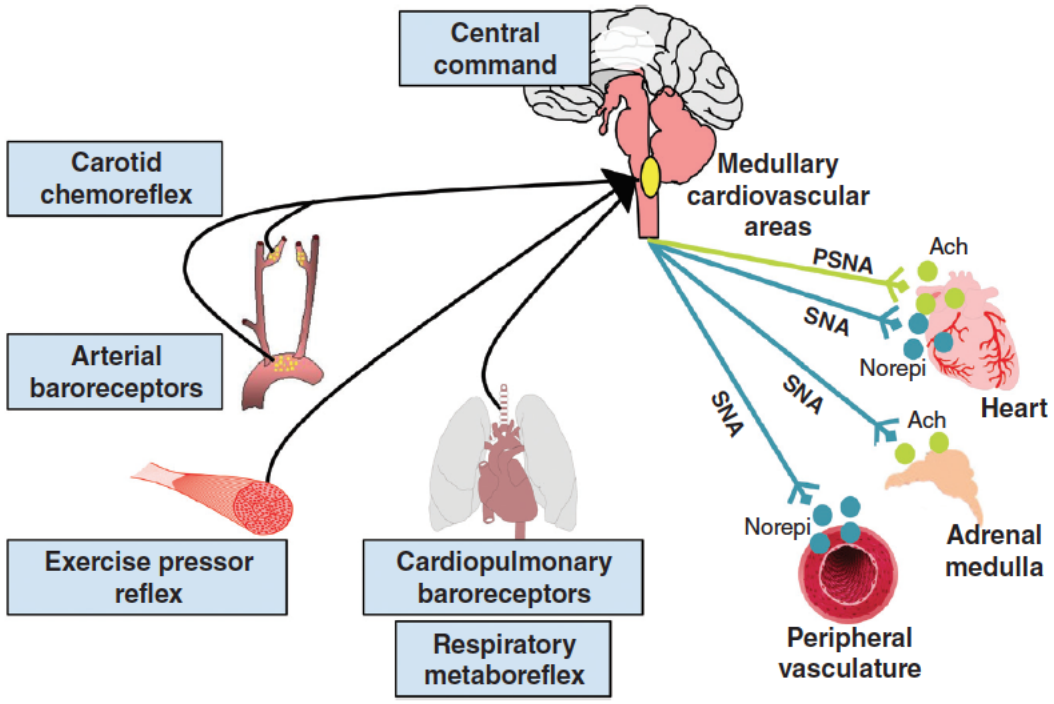

Figure 1-2 The regulation of the autonomic nervous system during exercise

At rest, carotid chemoreflex, arterial baroreceptors and cardiopulmonary baroreceptors send feedback signals to the cardiovascular control centres in the brainstem that mediate efferent SNS and PSNS activity. During exercise, feed-forward signals (central command), feedback from the carotid chemoreflex, arterial baroreceptors, metabolically and mechanically sensitive afferents in the skeletal muscle (exercise pressor reflex) and cardiopulmonary baroreceptors send neural signals to the brain altering the outflow of sympathetic and parasympathetic nerve activity to the periphery [from Fisher, Young and Fadel (2016)]. PSNA, parasympathetic nervous system; SNA, sympathetic nerve activity; norepi, noradrenaline; Ach, achetlycholine.

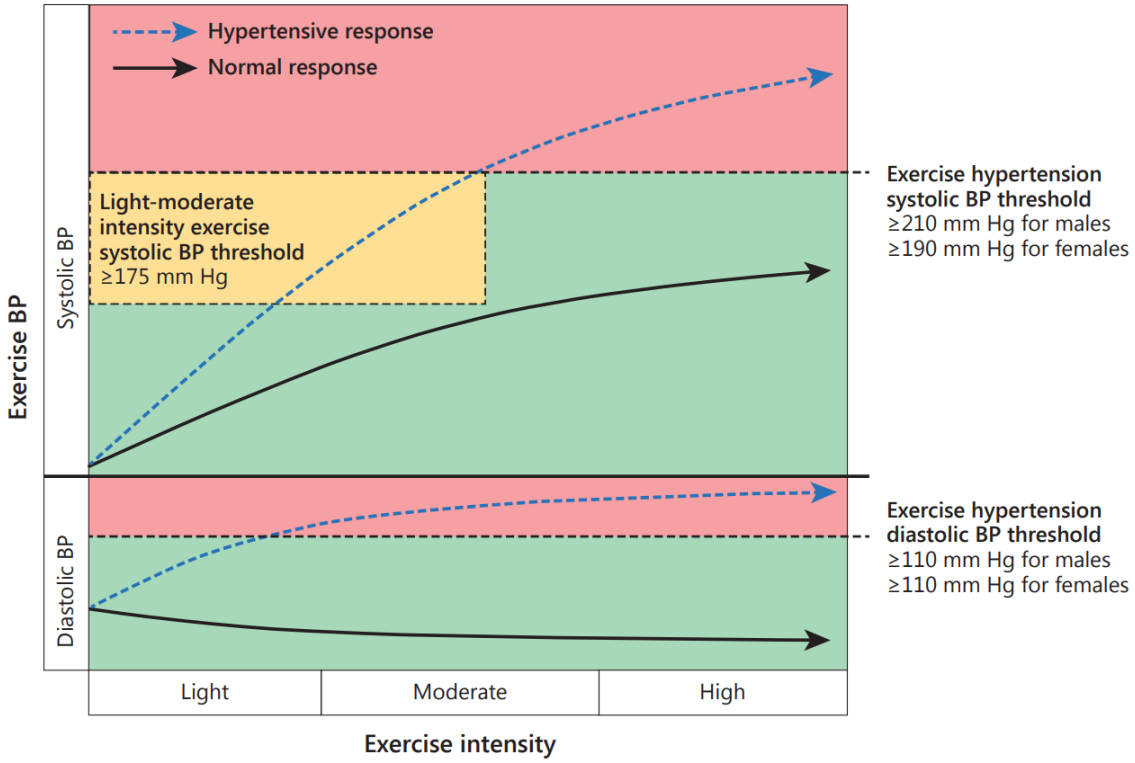

Figure 1-3 The normotensive and hypertensive response to exercise. Illustration demonstrating the difference between a normotensive response to exercise (black solid line) and a blood pressure response to exercise typical of a patient with hypertension (exercise hypertension) (dotted blue line). In normotensive individuals, systolic blood pressure increases gradually during increasing exercise intensities whilst diastolic blood pressure remains similar or drops below resting values. In contrast, in a typical patient with hypertension, systolic and diastolic blood pressure rise dramatically during increasing exercise intensities [from Schultz and Sharman, 2013).

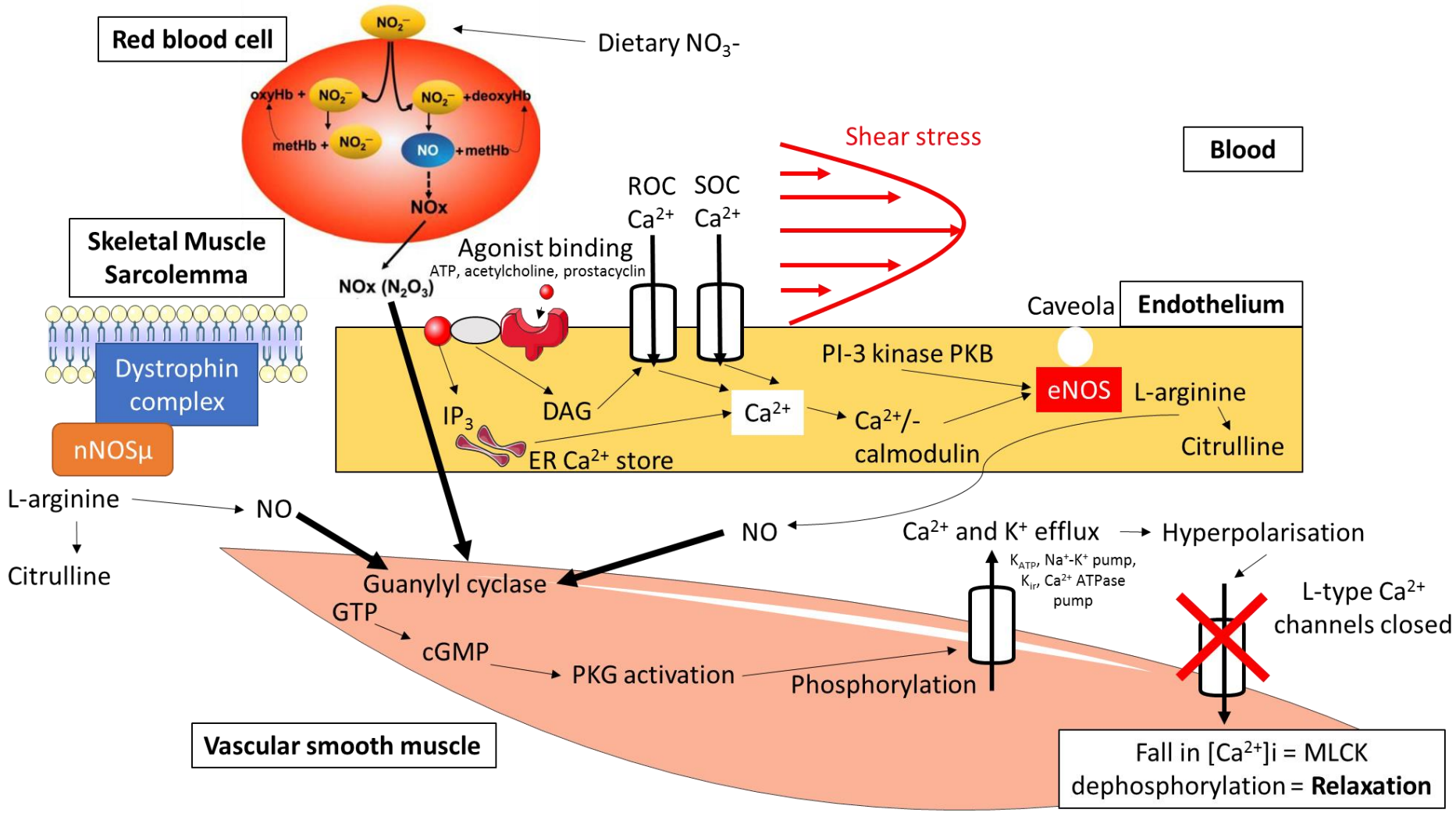
Red blood cell
NO_2^-
Dietary NO_3-
oxyHb +
+deoxyHb
metHb +
NO
+metHb
NOx
Shear stress
Blood
Skeletal Muscle Sarcolemma
ROC Ca^{2+}
SOC Ca^{2+}
Agonist binding
ATP, acetylcholine, prostacyclin
NOx (N_2O_3)
Caveola
Endothelium
Dystrophin complex
nNOSμ
PI-3 kinase PKB
eNOS
L-arginine
Citrulline
IP_3
DAG
Ca^{2+}
Ca^{2+}/-calmodulin
ER Ca^{2+} store
L-arginine
NO
Citrulline
NO
Ca^{2+} and K^+ efflux
Hyperpolarisation
K_{ATP}, Na^+-K^+ pump, K_{ir}, Ca^{2+} ATPase pump
L-type Ca^{2+} channels closed
Guanylyl cyclase
GTP
cGMP
PKG activation
Phosphorylation
Vascular smooth muscle
Fall in $[Ca^{2+}]i$ = MLCK dephosphorylation = **Relaxation**

Figure 1-4 A schematic of the regulation of nitric oxide during exercise. Nitric oxide is important in the regulation of blood flow during exercise and is regulated by increases in shear stress, agonist binding to the endothelium, nitrite reduction to nitric oxide and release from the skeletal muscle sarcolemma. In patients with hypertension, production of nitric oxide by the endothelium is impaired. Increasing nitrate bioavailability using beetroot juice increases nitrate-nitrite-nitric oxide reduction and may improve blood flow during exercise in patients with hypertension. NO_3^-; nitrate, NO_2^-; nitrite; oxyHb; oxygenated haemoglobin, metHb; methemoglobin, deoxyHb; deoxygenated haemoglobin, N_2O_3; dinitrogen trioxide, ATP; adenosine triphosphate, IP_3; inositol triphosphate, ER; endoplasmic reticulum, DAG; Diacylglycerol, ROC; receptor operated channels, SOC; store operated channels, Ca^{2+}; calcium, PKB; protein kinase B, eNOS; endothelial nitric oxide synthase, NO; nitric oxide, nNOSμ; sarcolemmal neuronal nitric oxide synthase, GTP; guanosine triphosphate, cGMP; cyclic guanosine monophosphate, PKG; protein kinase G, K^+; potassium, K_{ATP}; ATP-sensitive potassium channels, Na^+-K^+ pump; sodium-potassium pump, K_{ir}; inward rectifying potassium channels, Ca^{2+} ATPase pump; calcium ATPase pump, $[Ca^{2+}]I$; intracellular calcium, MLCK; myosin light chain.

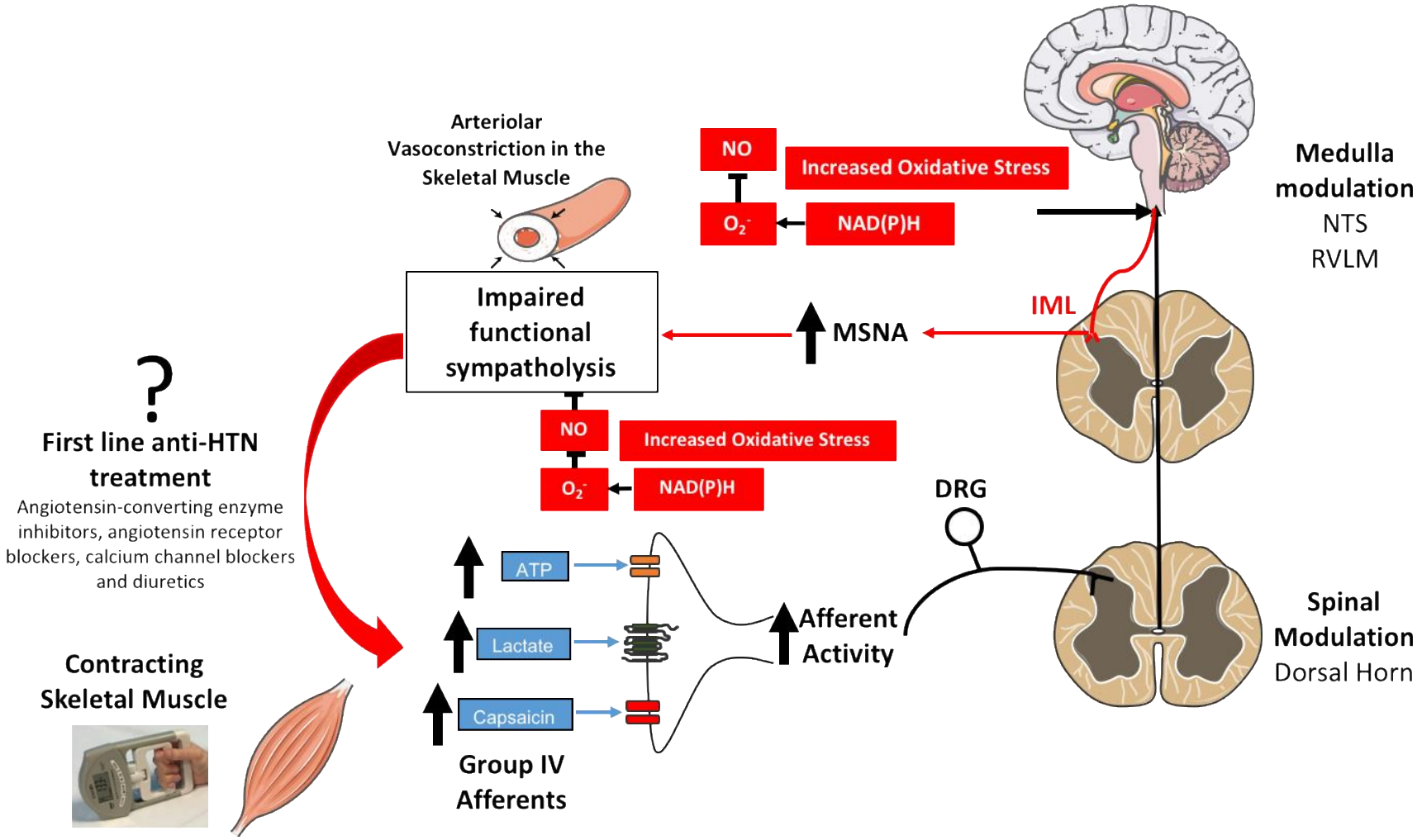
Arteriolar
Vasoconstriction in the
Skeletal Muscle
NO
Increased Oxidative Stress
O_2^-
NAD(P)H
Medulla
modulation
NTS
RVLM
IML
Impaired
functional
sympatholysis
MSNA
?
First line anti-HTN
treatment
Angiotensin-converting enzyme
inhibitors, angiotensin receptor
blockers, calcium channel blockers
and diuretics
NO
Increased Oxidative Stress
O_2^-
NAD(P)H
DRG
ATP
Lactate
Capsaicin
Afferent
Activity
Spinal
Modulation
Dorsal Horn
Contracting
Skeletal Muscle
Group IV
Afferents

Figure 1-5 A schematic illustrating the potential mechanism for metaboreflex hyperreflexia in patients with hypertension. Increased oxidative stress (red boxes) in the skeletal muscle leads to impaired functional sympatholysis in hypertension and this increases the level of metabolites (blue boxes) in the skeletal muscle which leads to increased metaboreflex hyperreflexia. The metaboreflex afferents are modulated at the dorsal root and further modulated supraspinally in the brainstem. Altered processing of the metaboreflex in the nucleus of solitary tract and rostral ventrolateral medulla leads to increased sympathetic nerve activity during exercise. Sympathetic nerve activity is normally offset in the skeletal muscle in healthy individuals, in patients with hypertension impaired functional sympatholysis causes a reduction in skeletal muscle blood flow. The exact location that mediates metaboreflex hyperreflexia in patients with hypertension is unclear but is likely multifactorial. The effect of first line treatment for hypertension on the metaboreflex and functional sympatholysis is unclear. DRG; dorsal root ganglion, NTN; nucleus of solitary tract, RVLM; rostral ventrolateral medulla, IML; intermediolateral cell column, NAD(P)H; nicotinamide-adenine dinucleotide phosphate oxidase, O_2^-; superoxide anions, NO; nitric oxide, MSNA; muscle sympathetic nerve activity, and ATP; adenosine triphosphate.

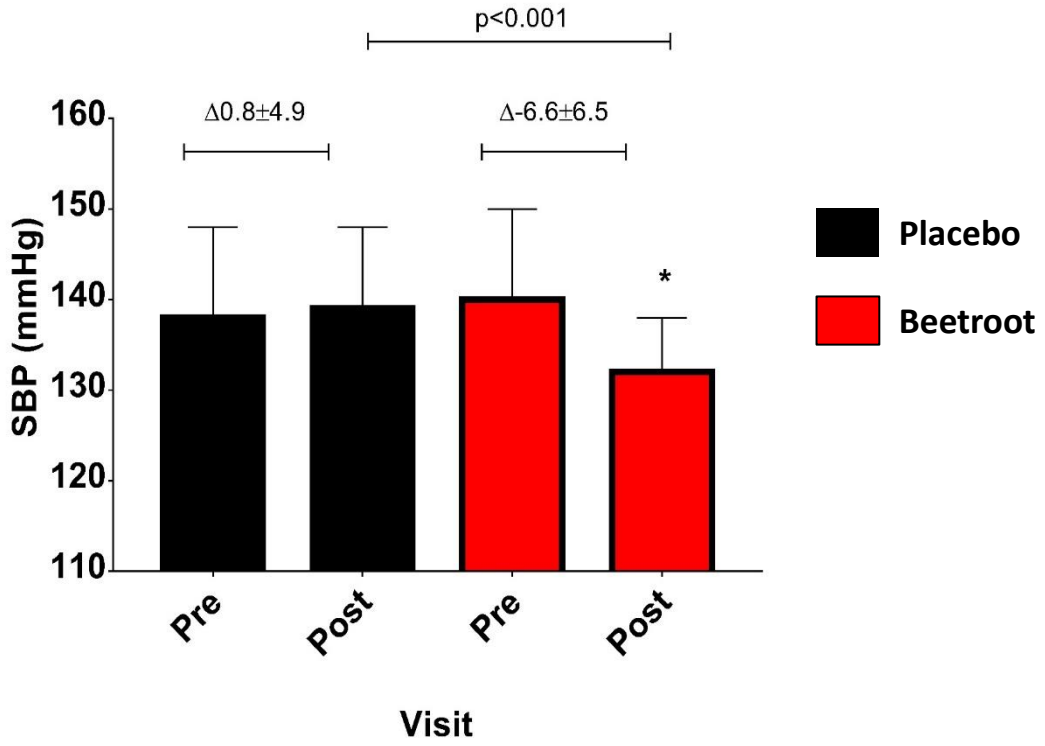

Figure 1-6 Dietary nitrate and ambulatory blood pressure in hypertension. Dietary nitrate intervention (red bars) for 4-weeks lowers 24-hour ambulatory SBP (SBP) in middle-aged male and female individuals with hypertension compared to a placebo (black bars). Figure from Kapil et al. (Kapil et al., 2015).

Chapter 2 General Methods

2.1 Participants

All of the studies in this thesis conformed to the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The procedures used were granted ethical approval by the Research Ethics Committee (REC) and the Health Research Authority (HRA). Ethical approval for the study in Chapter 3 and 4 was granted by the Southwest-Exeter NHS REC (16/SW/0004). The study in Chapter 5 was given ethical approval through the Northern-Ireland proportionate review NHS REC (17/NI/0097). As defined by the National Institute for Health and Care Excellence (NICE guidelines, 2011) normotension was defined as clinic blood pressure (BP) <140 mmHg and day-time ambulatory blood pressure (ABPM) <135/85 mmHg with the absence of anti-hypertensive medication. Treated-uncontrolled hypertension was defined as daytime ABPM ≥135/85 mmHg despite the use of at least one anti-hypertensive medication. Treated-controlled hypertension was defined as previous diagnosis of hypertension, but BP currently controlled with at least one anti-hypertensive therapy and daytime ABPM <135 mmHg. Finally, untreated hypertension was defined as daytime ambulatory BP ≥135 mmHg with the absence of anti-hypertensive medication.

General exclusion criteria for ***all*** studies included:

i) diagnosed major respiratory-cardiovascular (CV) disorders (i.e., severe cardiac electrical conduction abnormalities, angina, chronic heart failure, and respiratory diseases, such as chronic obstructive pulmonary disease).

ii) metabolic and endocrine disorders such as thyroid underactivity/overactivity, diabetes mellitus (type I and II), hyperlipidaemia and osteoporosis.

iii) major illness such as cancer, inflammatory disease including vasculitis, rheumatoid arthritis etc.

iv) pregnancy in women of child bearing age.

v) intravenous drug use and/or alcohol intake >28 units per week.

vi) taking nitrates/steroids/immunosuppressants.

vii) febrile illness within one week of participation.

viii) currently enrolled in a clinical trial of a medicinal product

ix) body mass index (BMI) >= 35 kg/m^2.

More specific exclusion criteria for each study can be seen in the respective Chapters. All participants attended the laboratory situated in the Clinical Research and Imaging Centre (CRiC)-Bristol at a similar time of day and lab conditions were controlled to a set temperature (22°C). Participants were asked to refrain from alcohol/caffeine consumption and strenuous exercise for 12 hours before the study visits. Participants were advised to have a small meal 2 or 3 hours prior to arrival at the laboratory. Participants were also asked to avoid the use of painkillers such as aspirin, paracetamol or anti-inflammatory drugs (e.g., ibuprofen) for 24-hours prior to the study visits. Participants were asked to refrain from these medications due to their known inhibitory effects on exercise BP (Drew et al., 2013, Cui et al., 2007, Cui et al., 2008b).

2.2 Procedures

2.2.1 Screening

Prior to all studies, participants were given a screening phone call to ensure that they met the specific inclusion criteria for the study and did not meet any of the exclusion criteria. Specific inclusion criteria for each study can be found in their respective Chapters. All participants gave written consent during the initial screening visit at the CRiC-Bristol. Participants also completed a screening questionnaire during the initial visit to rule out any of the exclusion criteria. Participants were then asked to rest for 10 minutes before resting clinical BP was taken using an automatic oscillometric monitor according to the European Society of Hypertension guidelines (Omron, 705IT, Omron Healthcare Europe) (O'Brien et al., 2001). The first reading was ignored and then a BP was taken on the left and right arm, with two further readings being taken on the arm where BP was highest (O'Brien et al., 2001). The average of these final two readings was taken as clinic BP. The participant also carried out a urine dipstick test (Siemens Multistix) to assess evidence of kidney damage and diabetes. Specifically, leukocytes, nitrite, urobilinogen, protein, pH, haemoglobin, specific gravity, ketone and glucose (Siemens Multistix) were measured. For females of a child bearing age, a human chorionic gonadotropin (hCG) hormone sensitive test was used for pregnancy screening. A 12-lead electrocardiogram (ECG) was completed by a research nurse and checked over by a Cardiologist to assess for any abnormalities. Any adverse incidents or events that occurred during the studies in this thesis were recorded in the study site files. Any serious adverse events were recorded and reported to the University of Bristol research and development governance team department and the UH Bristol research and development team.

2.3 Experimental Measures

2.3.1 Cardiopulmonary exercise testing

The peak volume of oxygen (O_2) that a participant could consume and use ($\dot{V}O_2$ peak) was assessed during all studies by an incremental exercise test on an upright cycle ergometer (Ergoselect 100, Love Medical, Manchester, UK). Prior to the $\dot{V}O_2$ peak test, a 12-lead ECG was fitted by a research nurse for continuous monitoring of heart rate (HR) and heart rhythm monitoring. $\dot{V}O_2$ peak was calculated by plotting workload (watts (W)) vs. O_2 uptake ($\dot{V}O_2$) and was expressed as mL/min/kg. The test began with a 5-minute baseline period while the participant was sat upright on the cycle ergometer and resting haemodynamics and respiratory values were assessed. The participants began cycling at 0 watts for 3 minutes. $\dot{V}O_2$ peak was assessed by using a ramp protocol of 25 watts per minute (25 watts/min) until volitional fatigue. Participants were asked to maintain a constant cadence of 60-80 revolutions per minute (RPM). $\dot{V}O_2$ max is defined as "the O_2 uptake during an exercise intensity at which actual O_2 intake reaches a maximum beyond which no increase in effort can raise it" (Hill et al., 1923). A plateau in $\dot{V}O_2$ is often not observed during maximal upright cycle ergometer exercise, as the addition of arm exercise at maximum cycle ergometer exercise increases $\dot{V}O_2$ (Taylor et al., 1955). $\dot{V}O_2$ peak is defined as the mean $\dot{V}O_2$ attained during the final 30 seconds of the exercise test and is used as a measure of peak exercise capacity from cycle ergometer testing (Wylie et al., 2016).

Prior to each $\dot{V}O_2$ peak test, the flow sensor (spirometry) used for gas and respiratory analysis (Ergostik CPET system, Love Medical, Manchester, UK) was

calibrated with high-precision calibration gases (5.13% carbon dioxide (CO_2), 15.15% O_2 and balance nitrogen) and a 3-litre calibration syringe. A facemask was fitted to each participant and checked for leaks prior to any testing. The facemask was fitted to a non-rebreathing valve. The $\dot{V}O_2$ was measured breath by breath which was determined by a flow sensor. The % O_2 inspired and expired was assessed by an electrochemical cell. More specifically, O_2 enters the flow sensor and comes into a contact with a cathode. O_2 then gets reduced to hydroxyl ions, which react with a lead anode and oxidise to lead oxide. These reactions cause the generation of a current which is proportional to the % of O_2 (Love Medical, Manchester, UK). The % CO_2 was determined by the flow sensor via infrared spectroscopy (Love Medical, Manchester, UK). Flow was measured by the flow sensor using the differential pressure principle (Love Medical, Manchester, UK). Tidal volume, breathing frequency, minute ventilation (VE), tidal volume, breaths per minute) were also measured using the Ergoflow flow sensor for spirometry (Ergostik CPET system, Love Medical, Manchester, UK). The VO_2 and V_ECO_2 were then calculated using the following equations:

VO_2 = (Inspired volume * Fraction of inspired O_2) - (Expired volume * Fraction of expired O_2) [equation 2.1]

V_ECO_2 = (Expired volume * Fraction of expired CO_2) - (Inspired volume * Fraction of inspired CO_2) [equation 2.2]

To ensure that participants had reached their $\dot{V}O_2$ peak the respiratory exchange ratio (RER) ($V_ECO_2/\dot{V}O_2$) was calculated. RER is used as an indicator of what the

body is using as its main energy source (fat or carbohydrate), at rest it is 0.7-0.8 where $\dot{V}O_2$ is higher than V_ECO_2 expired, indicating predominant fatty acid oxidation. However, during intense exercise V_ECO_2 increases due to increased CO_2 production from the active muscle and more O_2 is extracted by the working muscle, meaning that RER will increase to above 1. The criteria used for $\dot{V}O_2$ peak were: 1) An RER of >1.15 (Issekutz et al., 1962), 2) HR >85% of maximal HR, defined as 220-age, was used as another criteria to indicate maximal exercise (Brown et al., 2002) and 3) a rating of perceived exertion >17 on the 6-20 Borg scale (Church et al., 2008). The anaerobic threshold is defined as an intensity of exercise at which uptake of O_2 uptake cannot account for the majority of energy production (Wasserman, 1986). Exercise above the anaerobic threshold is associated with exponential increases in blood lactate (Wasserman, 1986). The anaerobic threshold was calculated by plotting a moving average of V_ECO_2 (L/min) against $\dot{V}O_2$ (L/min) to smooth out any random breath by breath fluctuations (Beaver et al., 1986). The intersection point for the V_ECO_2 and $\dot{V}O_2$ was regarded as the anaerobic threshold and is given as a percentage of $\dot{V}O_2$ peak (V-slope method) (Svedahl and MacIntosh, 2003, Sue et al., 1988, Schneider et al., 1993). Importantly, the V-slope method has been shown to correspond with increases in lactate above the lactate threshold and also the estimated bicarbonate threshold (Beaver et al., 1986).

In addition, the ventilatory efficiency slopes (VE/V_ECO_2 slope), defined as the relationship between minute ventilation and carbon dioxide production were measured (Rausch et al., 2013). A V_ECO_2 slope >34 has been shown to highlight high risk pulmonary hypertension and heart failure patients (Rausch et al., 2013,

Bard et al., 2006). However, little is known about the VE/V_ECO_2 slope in patients with essential hypertension, treated or untreated. The VE/V_ECO_2 slope is assessed by plotting VE against V_ECO_2 from the onset of exercise to peak exercise or baseline to the anaerobic threshold (Bard et al., 2006). One study found that there was a significant correlation between the VE/V_ECO_2 slope measured from baseline to peak and to anaerobic threshold (r=0.83) (Metra et al., 1992). However, for the studies in this thesis the peak VE/V_ECO_2 slope was used as this has increased prognostic value when compared to the VE/V_ECO_2 slope up to the anaerobic threshold (Bard et al., 2006). In addition, the peak VE/V_ECO_2 slope remained predictive of mortality in this large group of heart failure patients when $\dot{V}O_2$ peak was added to a cox regression model (Bard et al., 2006).

2.3.2 Assessment of the BP response to incremental exercise

Prior to $\dot{V}O_2$ peak testing the participants arm size was measured to ensure the correct cuff size was fitted to the participant. During the $\dot{V}O_2$ peak testing the participant was asked to drop their arm by their side as this helped increase the number of BP readings attained. The rise in BP during the $\dot{V}O_2$ peak test was assessed using an automated sphygmomanometer specific for exercise every 1.5 minutes (Love Medical, Manchester, UK). BP was measured every 1.5 minutes so that enough readings could be taken to assess the BP rise during each predefined percentage of $\dot{V}O_2$ peak testing (Baseline, 0-25%, 26-50%, 51-75%, 76-100% and peak $\dot{V}O_2$ peak testing) (Love Medical, Manchester, UK). During pilot testing, BP readings every 1 minute were attempted but participants found it uncomfortable to do this at this frequency. For isometric handgrip testing, beat-to-beat BP was measured using the Finapres (Finometer, FMS,

Netherlands; see section 2.6, page 108). The Finapres was not used to measure the BP response to peak exercise testing ($\dot{V}O_2$ peak test) as the Finapres is not validated for exercise, especially at high intensity (Finometer, FMS, Netherlands; see section 2.6) (Parati et al., 1989). Additionally, the Finapres measures BP from the finger and the hand needs to be very still for the Finapres to get a successful reading (Finometer, FMS, Netherlands; see section 2.6, page 108). This makes using the Finapres difficult during exercise. A 12-lead ECG (Love Medical, Manchester, UK) was used to measure HR during the $\dot{V}O_2$ peak test.

2.3.3 Repeatability of peak exercise blood pressure (within subjects)

A mixture of 9 treated controlled (n=5), uncontrolled (n=3) and untreated (n=1) individuals with hypertension from the study detailed in Chapter 3 came back and repeated a $\dot{V}O_2$ peak test. The participants returned to the laboratory between 2 weeks and 1 year following the study visit $\dot{V}O_2$ peak test. All patients with treated hypertension that came back remained on the same anti-hypertensive medications as they did when they came in for the study in Chapter 3. The repeat test results for absolute SBP and the change in absolute SBP can be found in Table 2.1 and 2.2 (page 125 and 126) and Figure 2.1 and 2.2 (page 134 and 135). From this small repeatability test it was found that the coefficient of variation for the absolute SBP during two $\dot{V}O_2$ peak tests is low (2.45%) (Table 2.1, page 125). There was a strong positive correlation found for the absolute SBP during $\dot{V}O_2$ peak testing (Pearson's r = 0.82; Figure 2.1, page 134). In addition, for the absolute change in SBP from baseline the coefficient of variation was 6.98% between two $\dot{V}O_2$ peak tests (Table 2.2, page 126). A strong positive correlation was found for the absolute change in SBP during $\dot{V}O_2$ peak testing (Pearson's r =

0.89; Figure 2.2, page 135). This suggests that the BP response to $\dot{V}O_2$ peak testing is reliable. These variations in SBP between tests will need to be considered in studies that are aiming to lower exercise SBP. For example, the change in absolute SBP during $\dot{V}O_2$ peak testing varies by about 6.98% and therefore effects of interventions looking to lower exercise SBP will have to have a larger effect than 6.98%. In addition, the $\dot{V}O_2$ peak score (ml/min/kg) remained similar between the two visits, a coefficient of variation of 6.07% was found (Table 2.3, page 127 and Figure 2.3, page 136). The coefficient of variation for $\dot{V}O_2$ peak scores was performed as changes in CV fitness between tests is likely to influence the BP response to exercise.

2.4 Arterial tonometry (Chapter 4)

Aortic BP and central (aortic) stiffness were measured using non-invasive pulse wave analysis and pulse wave velocity in participants with hypertension and normotension in the study detailed in Chapter 4.

2.4.1 Introduction to the arterial pulse pressure

The left ventricle ejects blood into the elastic aorta at a faster velocity than blood can drain away and the resulting increase of the volume of blood into the aorta leads to a steep increase in BP during systole. Only 20-30% of the blood ejected by the left ventricle goes to the peripheral vessels, while 70-80% is stored as mechanical energy in the elastic vessels. When the vessels recoil this mechanical energy is converted into pressure energy during diastole and helps to maintain perfusion (Windkessel effect). In young healthy individuals the Windkessel effect

helps the dampening of the PP (SBP-DBP) over the cardiac cycle whilst maintaining perfusion following left ventricular ejection. If the arteries had completely stiff walls the BP would be instantly increased throughout the whole arterial bed during systole due to a rapid pulse transmission velocity. Pulse wave transmission velocity travels at around 4-5 m/s in young healthy individuals and travels faster than the blood velocity which travels only at around 0.2 m/s in the ascending aorta. As the pulse wave transmission is dependent on arterial wall deformation, increased stiffness of the arteries increases transmission velocity of the pulse. Central (aortic) stiffness can be measured non-invasively using pulse wave analysis and pulse wave velocity. Both of these methods are discussed below. Central (aortic) stiffness was measured for the study detailed in Chapter 4 as elevated aortic stiffness has been shown to be related to an exaggerated peripheral BP response to exercise (Tsioufis et al., 2008, Thanassoulis et al., 2012).

2.4.1.1 Pulse wave velocity

The SphygmoCor System (AtCor Medical, Sydney SpA) was used as a non-invasive method for assessing pulse wave velocity as a marker of central (aortic) stiffness in participants for the study in Chapter 4. Pulse wave velocity is measured by assessing the transmission time of the R wave (as measured by a 3-lead ECG) to the onset of the pulse pressure wave in the 2 sites measured (see Figure 2.4, page 137). Further, this is then divided by the difference in the distance between the suprasternal notch and the 2 sites assessed (see Figure 2.4, page 137):

$$PWV = ds_a\text{-}ds_b/tt_a\text{-}tt_b \quad \text{[equation 2.3]}$$

d = distance

tt = pulse transmit time

s = suprasternal notch

The concept of pulse wave velocity is based on the Moens Korteweg equation (Bramwell and Hill, 1922). Velocity of the pulse wave is influenced by the radius, wall thickness, density of fluid and the elastic properties of the arteries (Messas et al., 2013):

PWV = $\sqrt{\text{Youngs}}\ \text{modulus} * \text{h}/2\text{R}\rho$ [equation 2.4]

PWV = pulse wave velocity

Youngs modulus represents the elastic properties of the artery for lateral expansion

h = wall thickness

R = radius

ρ = density of fluid

For thick walled tubes with flow the Moens Korteweg equation has been adapted to account for the assumption that pulse wave convection changes with the cross-sectional averaged velocity of the blood (Khir et al., 2001):

PWV = $(\sqrt{\text{Youngs}}\ \text{modulus} * \frac{\text{h}}{2\rho\left(\text{ri}+\frac{\text{h}}{2}\right)}) + U$ [equation 2.5]

ri = internal radius of the artery.

U = cross-sectional averaged velocity

It has been documented that decreases in the Youngs modulus, the elasticity of the artery, will cause an elevated pulse wave velocity (Khir et al., 2001). Carotid-femoral pulse wave velocity was assessed as this is the most established method for assessing aortic pulse wave velocity and arterial stiffness over carotid-brachial arteries (Tillin et al., 2007, Boutouyrie et al., 2002). In addition, the major advantage of using carotid-femoral pulse wave velocity as compared to the femoral-tibial or carotid-radial pulse is that propagation time is assessed along the aortic and aortio-iliac pathway (Laurent et al., 2006). This makes the assessment of the carotid-femoral pulse wave velocity the most clinically relevant as the ascending aorta and the common iliac artery are where the left ventricle first ejects blood into and are the most pathophysiological areas for the blood vessels to stiffen (Laurent et al., 2006). Pulse wave velocity has been shown to predict adverse CV events in the general population (Willum-Hansen et al., 2006, Mattace-Raso et al., 2006) and is elevated in hypertensive individuals and remains predictive of adverse CV outcomes (Blacher et al., 1999, Boutouyrie et al., 2002, Laurent et al., 2001, Laurent et al., 2003, Hua et al., 2005). The Framingham Heart Study found that in 2232 individuals, a carotid-femoral pulse wave velocity score of more than 11.8 m/s was an independent risk factor of adverse CV events (Mitchell et al., 2010). Importantly, central (aortic) pulse wave velocity as measured by the SpygmoCor (SpygmoCor System, AtCor Medical, Sydney) system has been validated, by comparing assessment of aortic pulse wave velocity during cardiac catherization (Weber et al., 2009) and via phase contrast magnetic resonance imaging (Hickson et al., 2010). This suggests that pulse wave velocity as measured by the SpygmoCor is an accurate measurement of central (aortic) pulse wave velocity.

Pulse wave velocity was calculated automatically by the SpygmoCor (SpygmoCor System, AtCor Medical, Sydney) using the equation (Laurent et al., 2006, Thanassoulis et al., 2012):

$$\text{Pulse Wave Velocity} = d_{PWV}/\Delta t \quad \text{[equation 2.6]}$$

where d_{PWV} = distance between sternal notch to femoral artery (cm) – distance between sternal notch carotid artery (cm) and Δt = the change in time.

2.5 Pulse wave analysis

2.5.1 Wave reflection and central blood pressures

Pulse wave analysis was also used as an estimate of central (aortic) BP using arterial tonometry. Higher central (aortic) BP is a marker of elevated central (aortic) stiffness (Pereira et al., 2013, Laurent et al., 2001, Laurent et al., 2006, Laurent et al., 2003).

SBP is typically measured in the brachial artery in clinical practice, however SBP is typically 40 mmHg higher in the brachial artery as compared to the aorta (Kroeker and Wood, 1955). This amplification of SBP towards the periphery arises mostly due to elevated arterial stiffness in the peripheral arteries as compared to the elastic aorta (McEniery et al., 2014) (Figure 2.5, page 138).

Two main models have been described to explain the SBP amplification towards the peripheral arteries. Firstly, the pressure wave transmitted by the central aorta

consists of a reflected wave back from the periphery (wave reflection) and a forward wave that is amplified out towards the peripheral organs. The forward wave is generated by the contraction of left ventricle during systole (Westerhof et al., 1972). Wave reflection is mediated by sites of impedance mismatch (e.g. bifurcations and high resistance arterioles are the major site of wave reflection in humans) (Hirata et al., 2006, Westerhof et al., 1972). The sites of impedance mismatch generate several wavelets during one cardiac cycle that summate into one reflected wave (McEniery et al., 2014). In healthy young and normotensive adults, the summed wave reflection doesn't influence aortic systolic pressure because the reflected wave returns during late diastole, there is high amplification of the pressure wave between the aorta and periphery, resulting in larger peripheral BPs compared to aortic (or central) BPs (Hirata et al., 2006). However, ageing is associated with an increased reflection of the pressure wave from the periphery, which occurs early in systole due to increased peripheral arterial stiffening which contributes to the aortic SBP and causes central BP to rise to a similar level as peripheral BP (Figure 2.4, 2.5, 2.6 and 2.7, pages 137-140) (Hirata et al., 2006, McEniery et al., 2014). This increases the load on the left ventricle and negatively effects ejection fraction and augments the myocardial O_2 requirements (Laurent et al., 2001). The presence of hypertension increases the reflection wave, compared to age matched normotensives, which raises aortic BP compared to age matched normotensives (Fantin et al., 2007).

A second paradigm that has been assessed to explain SBP amplification is an adaptation of the Windkessel model. The original two-element Windkessel model shows that during systole there is an ejection of blood from the left ventricle into a

compliant aorta. In the elastic arteries some of this energy is stored as potential energy and during diastole where the vessels this potential energy is converted into pressure energy. However this model fails to allow the investigation of the pulse wave transmission and SBP amplification in the periphery (McEniery et al., 2014). The adaptation of the Windkessel approach is a time-based model which accounts for both the central reservoir and the wave transmission functions of the CV system. The central reservoir is the potential energy stored following systole, before recoil during diastole (Wang et al., 2003). The model consists of the central reservoir pressure and an excess pressure:

> Excess pressure = aortic pressure – Windkessel pressure (reservoir pressure) [equation 2.7]

Excess pressure is defined as the pressure difference driving flow into the ascending aorta (Windkessel) (Wang et al., 2003). Aortic flow into the Windkessel is proportional to excess pressure under normal conditions, which suggests that the reflected wave contributes very little to the augmentation of peripheral SBP and central SBP under normal conditions (Wang et al., 2003). This model further describes how aortic flow, aortic pressure and peripheral SBP augmentation under normal conditions is mediated mostly by a forward wave (aortic outflow) and a reservoir pressure (Windkessel pressure) (Wang et al., 2003). Aortic BP was used for the study in Chapter 4 as an indication of central (aortic) stiffness.

Higher aortic BP is an indicator of elevated Windkessel stiffness in the aorta (Wang et al., 2003). Elevated aortic BP at rest, similar to elevated central (aortic) PWV

has been shown to be predictive of an exaggerated peripheral BP response to exercise (Thanassoulis et al., 2012).

To perform applanation tonometry using SphygomoCor (SpygmoCor System, AtCor Medical, Sydney) the participant was first asked to lay supine for 10 minutes of quiet rest. The participants resting BP was measured in the supine position from the brachial artery. In accordance with the European Society of Hypertension guidelines resting BP was assessed using an automatic oscillometric monitor (Omron, 705IT, Omron Healthcare Europe). The first reading was ignored and then a BP was taken on the left and right arm, with two further readings being taken on the arm where BP was highest (O'Brien et al., 2001). The average of these final two readings was taken as BP (O'Brien et al., 2001). This reading was then entered into the SpygmoCor System (AtCor Medical, Sydney). The system uses a BP reading from the periphery (brachial artery) to calibrate to aortic BP. To perform this analysis, a tonometer was placed over the radial artery for a sufficient period of time so that a radial pulse trace could be recorded. The SphygomoCor (SpygmoCor System, AtCor Medical, Sydney) system has an inbuilt calculation that assesses the variability of the recording, this is called the operator index and is a scale of 0-100 (100 being the best quality). If the operator index was <= 95 a repeat reading was done until the score was > 95. The SpygmoCor (SpygmoCor System, AtCor Medical, Sydney) systems assessment of central BPs is built on a general transfer function (Karamanoglu et al., 1993). The general transfer function is based on common frequency components of the peripheral pressure waveforms and the aortic pressure waveforms (Butlin and Qasem, 2017, Karamanoglu et al., 1993). The transfer function uses discrete Fourier transformation which is a

mathematical algorithm that creates a aortic BP waveform based on the extraction of simple sine waves of varying frequency and amplitude from the peripheral pulse in the time domain to harmonics in the frequency domain (Olafiranye et al., 2011). Several factors can influence the recreation of the aortic BP waveform, including, HR, blood volume, viscosity, vascular impedance and arterial compliance which the transfer function considers (Olafiranye et al., 2011, Karamanoglu et al., 1993). Importantly, the transfer function has been well validated against invasive aortic BP measurement by micromanometer (Chen et al., 1997, Pauca et al., 2001). Suggesting that the recreation of the central (aortic) BP wave from the radial artery is an accurate measurement of central (aortic) BP and aortic stiffness. From the recreated central pressure waveform, the software calculates aortic augmentation pressure (which is defined as the difference between the peak 1 and peak 2 wave in the central pressure waveform), aortic SBP, aortic PP and augmentation index (AIx %) (aortic augmentation pressure/aortic PP * 100) (Figure 2.7, page 140). The AIx was calculated as follows:

Aortic AIx (%) = aortic augmentation pressure/aortic pulse pressure*100

[equation 2.8]

where augmentation pressure is the magnitude of wave reflection which increases with reduced compliance of the elastic arteries seen in hypertension and aortic pulse pressure = aortic SBP – aortic DBP. The AIx % is taken as a marker of wave reflection (Hirata et al., 2006). AIx is also reported as AIx 75 which is normalised to a HR of 75, which allows the comparison between individuals with different resting HR. Pulse wave analysis was performed two times in each participant and the mean value is presented in Chapter 4.

2.5.2 Repeatability of carotid-femoral pulse wave velocity (within subjects)

A mixture of 7 normotensive (n=2), controlled (n=1), uncontrolled (n=2) and untreated (n=2) hypertensive individuals had their pulse wave velocity (carotid-femoral) measured on two separate occasions. The pulse wave velocity assessment was at least 2 weeks apart and no longer than a month was left between readings. All patients with treated hypertension that came back remained on the same anti-hypertensive medications as they did when they came in for the first assessment. The repeat rest results for pulse wave velocity can be found in Table 2.4 (page 128) and Figure 2.8 (page 141). From this small repeatability test it was found that the coefficient of variation for pulse wave velocity was 3.2% (Table 2.4, page 128). There was a significant strong positive correlation for pulse wave velocity (Pearson's r = 0.99, P=<0.0001; Figure 2.8, page 141). This suggests that my assessment of pulse wave velocity within individuals over 2 weeks to a month is reliable.

2.6 Handgrip exercise and metaboreflex assessment

In the relevant studies in this body of work, the sensitivity of the metaboreflex was assessed using circulatory occlusion following isometric handgrip exercise (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014). This is called post exercise ischemia (PEI) and is routinely used to assess the contribution of the metaboreflex to exercise BP and SNA (Delaney et al., 2010, Greaney et al., 2014, Sausen et al., 2009). PEI has been used extensively in the literature to isolate the metaboreflex since Alam and Smirk (1937) found that circulatory occlusion following forearm exercise causes SBP to be maintained at exercise levels when compared to no circulatory occlusion following the cessation of exercise.

Subsequent research by Kaufman et al. (1984) found that PEI in cats caused a higher percentage of group IV afferents to be activated compared to the group III afferents. Importantly, this PEI period appears to selectively isolate the metaboreflex, independent of the mechanoreflex and feed-forward central command (Kaufman et al., 1983, Kaufman et al., 1984).

Prior to isometric handgrip exercise, participants maximal voluntary contraction (MVC) was measured. Participants performed three maximal contractions of the handgrip dynamometer with the dominant arm with one minute between each attempt. The MVC was taken as the highest score achieved from the three measurements (Delaney et al., 2010). For the studies in Chapter 3 and 4, participants first performed isometric handgrip exercise at 30% of MVC for 1 minute. At 1-minute, isometric handgrip ended and an occlusion cuff was pumped up to 240 mmHg in all participants and this cuff remained pumped up for 1 minute 30 seconds (Delaney et al., 2010). This 1 minute 30 seconds of occlusion following isometric handgrip exercise was the PEI period (Figure 3.4, page 199). For the study in Chapter 5, the intensity of isometric handgrip was increased to 40% MVC for 1 minute and PEI was increased to 2 minutes. The rationale for this was that at an increased % of MVC more metabolites in the forearm will increase the activation of the metaboreflex (Crisafulli et al., 2006). Typical CV responses to activation of the metaboreflex include increases in muscle sympathetic nerve activity (MSNA), BP, HR, stroke volume (SV), CO and increased respiration (Amann et al., 2011a, Amann et al., 2010, Delaney et al., 2010). An important component of activation of the metaboreflex is an increased BP to perfuse active skeletal muscle (Amann et al., 2011a, Amann et al., 2010). However, the technique chosen to activate the

metaboreflex (exercise ischemia vs. PEI) appears to determine how the metaboreflex increase BP (Crisafulli et al., 2011). In a recent study, Crisafulli et al. (2011) showed that in healthy volunteers, activation of the metaboreflex increased BP via increasing CO. When the metaboreflex was isolated during PEI the increase in CO was driven by an increase in SV (Crisafulli et al., 2011). Interestingly, HR returned to resting levels during PEI following dynamic and static exercise with a small muscle mass (e.g., forearms) (Crisafulli et al., 2011, Watanabe et al., 2010, Fisher et al., 2013).

2.6.1 Haemodynamic measurements during baseline, isometric handgrip exercise and metaboreflex isolation

Participants rested for at least 15 minutes before a baseline period of 10 minutes was commenced. BP was measured from the opposite (non-exercising) arm during isometric handgrip exercise and PEI from the baseline period on a beat-to-beat basis using finger photo-plethysmography (Finometer, FMS, Netherlands; see section 2.6, page 108). A beat-to-beat estimate of SV was measured by the Finapres (see section 2.6, page 108). HR was recorded using a 3-lead ECG. To ensure that participants maintained normal breathing rates (eupnoea) during baseline, isometric handgrip exercise and PEI, a respiratory belt was used to measure respiratory rate. If the participant temporarily ceased breathing (apnoea) during isometric handgrip exercise the isometric handgrip test was repeated after a 10-minute recovery period.

2.6.2 Finapres

The Finapres system was used to measure beat-to-beat SBP and DBP during baseline, isometric handgrip exercise and PEI. The Finapres system uses the volume clamp protocol of Penaz (1973). Using infrared photoplethysmography (light) from an inflatable finger cuff (Figure 2.9, page 124) the volume of blood and artery size is continuously measured. Owing to the non-linear relationship between transmural pressure and volume, the diameter of the artery is clamped to a 'setpoint' which occurs despite changes in arterial pressure during the cardiac cycle (Boehmer, 1987). This 'setpoint is accomplished via a counter pressure from an inflatable finger cuff that applies an external pressure to the finger equal to the pressure inside of the arterial wall (Boehmer, 1987). This keeps transmural pressure (transmural pressure = arterial pressure – external pressure) at zero (Boehmer, 1987). As the blood volume in the finger is determined by light intensity, an increase in light intensity indicates a drop-in blood volume and the cuff pressure is reduced by a rapid servo-controller system to allow blood volume to increase which allows light intensity to return to the set point. The opposite occurs when light intensity declines. At zero transmural pressure the external counter pressure by the finger cuff is equal to intra-arterial pressure in the finger (Imholz et al., 1988). At zero transmural pressure the veins in the finger are fully collapsed, but the arteries maintain a third of their cross-sectional area and volume, the arteries are said to be 'unloaded' and are maintained at zero transmural pressure (unstressed diameter) (Imholz et al., 1988). Therefore, during systole blood flow continues to flow out of the finger and during diastole flow continues to flow into the finger, maintaining oxygenation levels to an normal level in the finger (Gravenstein et al., 1985). Once per minute

(up to 70 seconds) the Finapres calibration system (PhysioCal) automatically checks for any changes in the unloaded artery size induced by smooth muscle vasoconstriction or vasodilation and rapidly adjusts the finger cuff pressure accordingly by altering the set point (Wesseling et al., 1995). The measurement of beat-to-beat SBP and DBP is temporarily interrupted during PhysioCal.

This technique produces a finger pressure waveform; however, finger artery pressure pulsations vary in shape and amplitude than pressures recorded from the clinical site of BP measurement in the brachial artery. The Finometer has built in software to account for these differences. Firstly, the Finometer Pro reconstructs the brachial artery pressure from the finger BP waveform using waveform filtering via an inbuilt transfer function (Guelen et al., 2003). The reconstructed waveform is similar in shape but not in magnitude (Bos et al., 1996). From the reconstructed waveform the pressure level differences between the finger and brachial arteries can be calculated using a level correction equation (Gizdulich et al., 1997, Bos et al., 1996). Finally, a return-to-flow systolic pressure was assessed via a standard Riva-Rocci cuff around the upper arm of the participant and is used to calibrate the waveform and level corrected pressures (Guelen et al., 2003). This is only performed once and is completed prior to any baseline recordings.

The Finometer Pro (Finometer, FMS, Netherlands) when measuring absolute SBP fails to meet the guidelines of the Advancement of Medical Instrumentation (AAMI) of a standard deviation of < 8 mmHg between intra-arterial BPs and the device being tested (Imholz et al., 1998). However, the change in SBP or DBP

using the Finometer Pro is reliable when measured at rest when compared to intra-arterial BP (Parati et al., 1989) and during tests that induce a pressor response, including handgrip exercise (Parati et al., 1989). Importantly, tracking the change in BP has been shown to be reliable in patients with hypertension compared to intra-arterial BP (Bos et al., 1992).

2.6.2.1 Modelflow method

The Modelflow method non-invasively assesses left ventricular SV by computing an aortic waveform using a three-component model of aortic input impedance (Wesseling et al., 1993). This model of aortic input impedance (Westerhof et al., 1971) describes the relationship between the aortic inflow and pressure by assessing the aortas opposition to left ventricular ejection (Westerhof et al., 1971). This model describes the relationship between aortic pressure and flow to give readings of cardiac SV and therefore enables the calculation of beat-to-beat CO (SV * HR) (Wesseling et al., 1993). The three components of the model flow method (Figure 2.10, page 143) are:

1. Aortic characteristic impedance (Z_0): Upon left ventricular contraction, blood is forced into the aorta but the aorta already contains a certain amount of blood and this existing pressure apposes left ventricular ejection. Z_0 is the resistance of the aorta to pulsatile inflow from the contracting left ventricle (Wesseling et al., 1993, Bogert and van Lieshout, 2005). Increases in pressure lead to minimal changes in Z_0.
2. Windkessel (buffer) compliance (C_W): This is the ability of the aorta to expand upon receiving blood during left ventricular contraction during systole. A highly compliant aorta will expand leading to minimal increase in

aortic pressure (Bogert and van Lieshout, 2005). An increase in pressure leads to a non-linear reduction in compliance. In a stiffer aorta a given increase in volume will lead to a larger increase in pressure.

3. TPR (R_p): overall resistance of the vascular bed which is influenced by a diverse range of factors, such as sympathetic activity, certain medications and metabolism (Wesseling et al., 1993).

The aortic impedance and Windkessel compliance are the two major determinants of systolic aortic inflow and are dependent on the elasticity of the aorta (Wesseling et al., 1993). The time course of changes in cross-sectional area of the aorta varies with pressure in a non-linear fashion. At low pressures, the cross-sectional area increases rapidly, whereas at higher pressures, the cross-sectional area changes slowly, as compliance is reduced (van Lieshout et al., 2003). The aortic cross-sectional area for pressure (A(P)) has been described as a mathematical equation:

$$A(P) = A_{max} [0.5+1/\pi \arctan (P - P0/P1)] \quad \text{[equation 2.9]}$$

where A_{max} is the maximal diameter of the aorta during ejection, P is pressure, P0 is the position of the inflection point on the pressure axis at 0.5 A_{max}, and P1 is the steepness of the curve at 0.75 A_{max}. As it is assumed that the aortic length (L) remains constant, the change in volume (V) (as assessed by $V = \pi r^2 L$ or $V=AL$, A being area and r being radius) is proportional to changes in the cross-sectional area of the aorta (Wesseling et al., 1993). Therefore, the two main components of systolic inflow are calculated as follows:

Windkessel compliance (C_w): the change in area (dA)/ the change in pressure (dP) [equation 2.10]

The aortic impedence (Z_0): √density of blood (*p)*/(Area (A) * Compliance (C)) [equation 2.11]

The values of C_w and Z_0 are computed once per beat. However, the values of P0, P1 and A_{max} during measurement are taken from an inbuilt database from a study that found that the values of aortic impedance and Windkessel compliance were related to age, gender, height and weight (Langewouters et al., 1984). The model parameters are simulated during recording which outputs a continuous aortic waveform (Figure 2.10, page 143). The waveforms are produced on a beat-to-beat basis and it is integrated during systole to give a measurement of SV (Figure 2.10, page 143).

A problem with the Modelflow method is that the A_{max} component has been shown to be variable between individuals, and the system assumes set values (Langewouters et al., 1984). This has caused considerable variation in the calculation of absolute SVs from model flow when compared to other methods such as Doppler Ultrasound (van Lieshout et al., 2003, Dyson et al., 2010) and thermodilution (Jansen et al., 2001). However, tracking the change in SV using the Finapres model flow method is tied to Doppler Ultrasound changes (van Lieshout et al., 2003) and thermodilution (Wesseling et al., 1993), although this finding isn't always consistent (Dyson et al., 2010). Indeed, initial calibrations of the model flow method to a gold standard method such as thermodilution is

needed for accurate measurements of absolute SV and CO (Bogert and van Lieshout, 2005). Nevertheless, changes in SV and CO have been reported as accurate compared to echocardiography during isometric exercise (van Dijk et al., 2005). For the studies in this thesis there was no capability to calibrate the Modelflow technique to a gold standard method and therefore changes in beat-to-beat SV are reported relative to a baseline period.

2.6.3 Reliability of the BP response to metaboreflex isolation measured via the Finapres

To assess the reliability of the BP response to metaboreflex isolation, test re-test data were collected from 8 people normotensive (n=1), treated controlled (n=5) and uncontrolled (n=2) hypertensives. More specifically, participants performed isometric handgrip exercise for 1 minute at 30% MVC and an occlusion cuff was pumped up to 240 mmHg for 1 minute 30 seconds (PEI). The time between repeat metaboreflex assessment was at least 2 weeks apart and no longer than a month was left between readings. All patients with treated hypertension that came back remained on the same anti-hypertensive medications as they did when they came in for the first assessment. The repeat rest results for metaboreflex can be found in Table 2.5 (page 129) and Figure 2.11 (page 144). From this small repeatability test it was found that the coefficient of variation for absolute change in SBP from baseline during PEI was 13% (Table 2.4, page 128). There was a significant strong positive correlation for the absolute change in SBP during PEI (Pearson's r = 0.90, P=<0.0021; Figure 2.11, page 144). Any interventions aiming to lower SBP during PEI will need to find a larger decrease

in SBP than 13% for it to be meaningful for a study with a similar number of participants.

2.7 Microneurography (Chapter 5)

Microneurography was used to measure acute and temporal changes in multi-unit MSNA in the peroneal nerve. Microneurography was measured to assess the level of MSNA at rest. The resting level of MSNA was used to compare to the level of MSNA during isometric handgrip exercise and during PEI. Microneurography was originally developed in Sweden by clinical neurophysiologists Karl-Erik Hagbarth and Ake Valbo between 1965-1966 and data from microneurography was first presented at a Scandinavian electrophalography (EEG) meeting in Copenhagen in 1966 (Vallbo and Hagbarth, 1967). Gunnar Wallin led the development of studying the measurement of sympathetic bursts to skin and muscle and it was first measured successfully in 1972 (Delius et al., 1972a, Delius et al., 1972b).

2.7.1 Methodology of microneurography

Microneurography measures changes in the post-ganglionic efferent unmyelinated C- fibres of the sympathetic nervous system. Efferent sympathetic nerve activity (SNA) can be measured from any peripheral nerve. However, the peroneal nerve in the leg, proximal to the fibular head is the most commonly used nerve to measure efferent SNA. Proximal to the fibula head, the peroneal nerve bifurcates into the deep and superficial portions. An obvious limitation to performing microneurography in the peroneal nerve is not being able to assess

MSNA during dynamic leg exercise, but it does allow measurement of MSNA during upper body exercise, such as isometric handgrip testing. Advantages are that it is easily identifiable, it is a peripheral nerve, the leg can be held still for a long time, and can be performed whilst the participant is supine, semi-supine or seated upright.

Participants were asked to position themselves semi-supine on the bed and the leg and foot were partially elevated and supported. The participant was then encouraged to fully relax their leg throughout the test to avoid activation of motor units. Next, cutaneous electrical stimulation was used to locate the position of the peroneal nerve. To stimulate the nerve, a blunt tipped stimulator, which applies a small electrical current ranging from 1-3 mAs for short durations (1 ms) was applied to the area surrounding the peroneal nerve. More specifically, electrical stimulation of the deep peroneal nerve leads to dorsiflexion and stimulation of the superficial portion leads to lateral movements of the foot (Vallbo et al., 1979). Where electrical stimulation lead to dorsiflexion, the stimulator was moved laterally and/or vertically to assess where the dorsiflexion was strongest. As this is an indicator of the position of the deep peroneal nerve (Vallbo et al., 1979), a small red dot was marked on the participants leg (Figure 2.12, page 145). At least 3 or 4 good sites with dorsiflexion are located before moving on to the next step (Figure 2.12, page 145).

The tip of a 35 mm un-insulated tungsten reference micro-electrode was then inserted into the participants skin around 1-2 cm from the expected site of the deep peroneal nerve. This acts as an electrical reference that allows changes in

electrical activity to be detected by the active micro-electrode. The reference micro-electrode is not normally moved until the end of recording. An un-insulated active tungsten micro-electrode (5μM tip diameter, shaft diameter of ~100-200 μM and impedance 2 MΩ at 1 kHz) was then inserted through the skin and into the nerve. Higher electrode impedances can also be used when the aim is to assess a smaller area of the nerve (e.g., single-fibre recording) (Macefield et al., 1994). Tungsten is used for microneurography as its electrical (conduction) and mechanical (non-brittle, thin and stiff) properties make it suitable for the percutaneous insertion into the nerve. The position of this active micro-electrode is moved until it is in a satisfactory position within the deep portion of the peroneal nerve that indicates MSNA.

Several criteria were used to assess whether a satisfactory MSNA signal had been obtained. Firstly, efferent sympathetic outflow in humans consists of either MSNA or skin sympathetic nerve activity (SSNA) (Delius et al., 1972a, Delius et al., 1972b). Multiunit MSNA is characterised by cardiac synchronicity and an increase in frequency of bursts during breath hold at the end of normal expiration (Wallin et al., 1973, Delius et al., 1972a, Delius et al., 1972b, Hagbarth et al., 1972). Another method for assessing whether MSNA was found was by assessing afferent activity. Nerve discharge when tapping the muscle belly of the anterior tibialis, activating the toe extensors and applying pressure to the tendon of the foot was also used as another indication of MSNA. In contrast to muscle nerves, changes in multiunit records of SSNA are evoked by alterations in arousal, environmental temperature, emotional status and by light stroking of the skin (Delius et al., 1972a, Delius et al., 1972b). Multiunit SSNA have no cardiac

rhythmicity (Hagbarth et al., 1972) (Figure 2.13, page 146). Increases in efferent SSNA are independent of changes in BP, unlike MSNA, suggesting a lack of baroreflex control over nerve activity to the skin (Hagbarth et al., 1972) (Figure 2.13, page 146). It is highly important to consider these classification factors when measuring MSNA as afferent and efferent nerves from the skin and muscle transverse within the same nerve. Having mixed MSNA and SSNA can lead to difficulties in detecting MSNA bursts during analysis (White et al., 2015). Once a successful site was obtained, participants were asked to lie quietly for a 10-minute baseline period prior to metaboreflex testing (see section 2.5, page 103).

The active and reference electrode were attached to a pre-amplifier that amplifies the signal before the signal reaches a main amplifier (80,000-fold amplification). The raw signal was band-pass filtered between 700 and 5000 Hz) (White et al., 2015). The amplified and band-pass filtered signal was then full-wave rectified and integrated (time constant 0.1s) using computer-based algorithms (Absolute Design and Manufacturing Services, Iowa). The raw and integrated signal were then displayed on a data acquisition software on a laptop (AD Instruments, LabChart Pro version 7). A 3:1 signal to noise ratio was used to assess whether the signal obtained was of good enough quality for analysis (Hart et al., 2017). To identify MSNA during baseline, isometric handgrip exercise and PEI a script written in a data analysis program was used (Spike 2, Cambridge Electronic Designs). MSNA is most commonly quantified as bursts per minute (burst frequency), which shows the amount of MSNA that the vascular smooth muscle is exposed to in a period of time, and also bursts per 100 heartbeats (burst incidence), which accounts for an individual's HR. Both methods for quantifying

MSNA are normally reported, MSNA bursts per 100 heartbeats should be interpreted with some degree of caution as if there are large increases in HR during a stressor (e.g., exercise) without a change in MSNA this would suggest a decrease in the level of MSNA (White et al., 2015).

Figure 2.14 (page 147) illustrates how challenging it is to attain a high quality MSNA signal in every individual (Tompkins et al., 2013). Figure 2.14 (page 147) also demonstrates that certain parts of the peroneal nerve contain larger concentrations of active recording sites for MSNA than others and how subtle adjustments in the position of the electrode can lead to larger or smaller burst size (Tompkins et al., 2013). In the peroneal nerve postganglionic sympathetic C-fibre fascicles, axons can exist singularly or in bundles, they are more commonly found in bundles of 2-42 (up to 44) axons (Tompkins et al., 2013, Macefield et al., 1994). Using a signal-to-noise ratio of >3 the smallest detectable action potential in the integrated trace is when four or more axons are firing (Salmanpour et al., 2011, Steinback et al., 2010). Some individuals have an increased number of large bundles of axons when compared to others and this enhances the ability to get a good quality recording in some individuals compared to others (Tompkins et al., 2013).

2.7.2 Identifying and quantifying MSNA (bursts/min, bursts/100Hb, MSNA area and total MSNA)

MSNA burst identification was completed using a script written in a data analysis program (Spike 2, Cambridge Electronic Designs). Firstly, a minimum amplitude was set for the detection of MSNA bursts, which was set for ~2 standard

deviations above the level of noise (Hart et al., 2017). The program then moves through the file and automatically determine bursts that are above the level of noise. The electrocardiogram signal is then marked (to calculate latency between R wave and the next burst). Next, the whole signal was manually assessed to ensure that the bursts were correctly identified by the program. The bursts are checked for their latency from the previous R wave, multiunit MSNA bursts typically have a latency of ~1.3s (Hart et al., 2017, Salmanpour et al., 2011). The latency of a multiunit MSNA is mediated by the size of the burst, with larger bursts having increased latency (Salmanpour et al., 2011). The latency was then plotted against burst amplitude to check that bursts have been marked correctly. If any bursts were identified as outliers the burst was identified and reviewed accordingly. Following this, the script automatically calculates burst frequency (bursts/min) and burst incidence (bursts/100Hb).

This analysis was completed for 10 minutes of baseline, isometric handgrip exercise, PEI and during 5 minutes of recovery for the study in Chapter 5. In addition, MSNA burst strength was assessed, this involves measuring MSNA burst area which was assessed by the same data analysis program (Spike 2, Cambridge Electronic Designs). Burst area was calculated by firstly assigning the largest spontaneous burst in the signal as 100 arbitrary units (AU) and a period of no bursts was marked as 0 AU (Hart et al., 2017). The start and end of each multiunit MSNA burst were then marked and the integral was then assessed between ‘start’ and ‘end’ (Hart et al., 2017). However, an issue with using this technique for analysis is that in one visit the needle may be close to the muscle sympathetic nerve, showing a large voltage on the trace, whereas in the next visit

the electrode could be further away from the nerve producing a smaller voltage. Baseline MSNA burst strength should therefore not be compared between two visits. Changes in MSNA burst strength will be reported as an absolute and percentage change (Fonkoue and Carter, 2015). Measuring MSNA area allows the total area to be calculated as the sum of burst area/time and the sum of burst area/HR to account for differences in HR (White et al., 2015).

2.7.3 Reliability of repeat microneurography assessments

2.7.3.1 Intra-observer reliability

Neurograms recorded from people with normotension (n=2) and treated controlled hypertension (n=4) were used to assess intra-observer reliability. Resting MSNA (bursts/min) and MSNA (bursts/100Hb) were analysed on 3 separate occasions with at least 24 hours between the analysis. The intra-observer reliability are shown in Table 2.6. and Table 2.7. (pages 130 and 131). The coefficient of variation for MSNA (burst/min) was 4% and MSNA (burst/100Hb) was 4% (Table 2.6 and 2.7, page 130 and 131).

2.7.3.2 Inter-observer reliability

To assess inter-observer reliability, neurograms from 4 different patients with treated-controlled hypertension were analysed on 3 separate occasions by two different observers. The files were analysed on 3 separate days with at least 24 hours between the analysis. The inter-observer reliability for the 4 treated controlled hypertensives results during baseline are in Table 2.8. and Table 2.9. (pages 132 to 133). From this small interobserver repeatability test it was found

that the coefficient of variation for MSNA (burst/min) was 4% and MSNA (burst/100Hb) was 4% between two observers (Table 2.8 and 2.9, pages 132 to 133).

2.8 General data analysis

All data was collected using a data acquisition system (LabChart 7 or 8, AD Instruments). Data were analysed using Spike 2 (Cambridge Electronic Designs), R studio version 3.4.1 (RStudio: Integrated Development Environment for R, Boston, MA) and LabChart 7 (AD Instruments). Analysed data were stored using Microsoft Excel (Microsoft Corp Redmond, WA). Statistical analysis was completed in IBM SPSS Statistics 24 (IBM Corp, Armonk, New York) and GraphPad version 7 (GraphPad Software, La Jolla California USA). Specific data analysis techniques will be discussed in more detail in the relevant Chapters.

2.9 Statistics

Power calculations for each study are provided in the relevant Chapter. Specific statistical tests performed will be discussed in detail in the relevant Chapters. Where relevant, averaged data are presented as mean ± standard deviation. Data were tested for normal distribution using a D'Agostino-Pearson omnibus K2 normality test. α was set at 0.05.

2.10 Tables

Table 2-1 Repeat assessments of peak absolute systolic blood pressure (SBP) during $\dot{V}O2$ peak testing on two different test days with no intervention.
The coefficient of variation indicates that the measurement of peak SBP during peak cycle ergometer exercise ($\dot{V}O_2$ peak testing) is repeatable (mean ± standard deviation).

Participant initials	**Test one Peak absolute SBP (mmHg)**	**Test two Peak absolute SBP (mmHg)**	**Coefficient of Variation (%)**
HP	206	212	2.02
KP	212	212	0
AH	183	185	0.77
EA	207	194	4.58
GW	227	194	11.09
NH	155	153	0.98
JM	208	207	0.34
KM	209	208	0.34
GS	213	219	1.96
Average	**202±21**	**198±20**	**2.45±3.53**

Table 2-2 Repeat assessments of the absolute change in peak systolic blood pressure (SBP) $\dot{V}O2$ peak testing on two different test days with no intervention. The coefficient of variation indicates that the measurement of peak absolute change in SBP during peak cycle ergometer exercise is repeatable (mean ± standard deviation).

Participant initials	**Test one peak absolute change in SBP (mmHg)**	**Test two peak absolute change in SBP (mmHg)**	**Coefficient of Variation (%)**
HP	81	92	8.99
KP	79	80	0.89
AH	65	57	9.27
EA	45	43	3.21
GW	85	68	15.71
NH	41	43	3.37
JM	91	82	7.36
KM	92	83	7.27
GS	80	88	6.73
Average	**73±19**	**71±19**	**6.98±4.33**

Table 2-3 Repeat assessments of $\dot{V}$O2 peak scores (ml/min/kg) on two days (mean ± standard deviation).

Participant initials	**Test one $\dot{V}O_2$ peak scores (ml/min/kg)**	**Test two $\dot{V}O_2$ peak scores (ml/min/kg)**	**Coefficient of Variation (%)**
HP	30	32.20	5
KP	23.03	23.80	5.46
AH	18	17.90	0.39
EA	10.40	10.20	1.37
GW	27.50	22.30	14.77
NH	22.80	25.60	8.18
JM	28.50	33.30	10.98
KM	20.20	21.25	3.58
GS	45	42	4.88
Average	**25.05±9.6**	**25.39±9.36**	**6.07±4.58**

Table 2-4 Repeat assessments of carotid-femoral pulse wave velocity (m/s) on two different test days with no intervention (mean ± standard deviation).

Participant initials	**Test one PWV (m/s)**	**Test two PWV (m/s)**	**Coefficient of Variation (%)**
AW	6.7	7.4	7
SBN	6.1	5.9	2.4
CW	7.9	7.6	2.7
MS	13.9	13.4	2.6
BT	16.5	15.6	4
ET	10.3	10.4	0.7
RG	11.4	10.9	3.2
Average	**10.4±3.8**	**10.2±3.5**	**3.2±1.9**

Table 2-5 Repeat assessments of the absolute change in SBP (mmHg) during PEI (PEI) on two different test days with no intervention (mean ± standard deviation).

Participant initials	**Test one absolute change in SBP (mmHg)**	**Test two absolute change in SBP (mmHg)**	**Coefficient of Variation (%)**
JR	35	40	9.43
KP	24	20	12.86
AH	26	31	12.40
GW	34	32	4.29
CJ	32	46	25.38
SHY	6	8	20.20
HP	26	31	12.40
DL	41	43	3.37
Average	**28±11**	**31±13**	**13±7**

Table 2-6 Repeat intra-observer analysis of bursts of muscle sympathetic nerve activity per minute (MSNA bursts/min) (mean ± standard deviation).

Participant initials	**Test one (MSNA bursts/min)**	**Test two (MSNA bursts/min)**	**Test three (MSNA bursts/min)**	**Coefficient of Variation (%)**
CC	45	42	45	4
JP	32	34	33	**3**
KP	42	42	43	1
AM	59	60	61	2
PR	36	35	33	4
GS	35	30	32	7
Average	**39±10**	**39±10**	**39±9**	**4±2**

Table 2-7 Repeat intra-observer analysis of bursts muscle sympathetic nerve activity per 100 heart beats (100Hb) (MSNA bursts/100Hb) (mean ± standard deviation).

Participant initials	**Test one (MSNA bursts/100Hb)**	**Test two (MSNA bursts/100Hb)**	**Test three (MSNA bursts/100Hb)**	**Coefficient of Variation (%)**
CC	78	72	78	4
JP	58	61	59	3
KP	79	79	80	1
AM	70	70	71	1
PR	76	74	78	4
GS	56	51	54	5
Average	**67±14**	**67±12**	**67±12**	**4±2**

Table 2-8 Repeat interobserver analysis of bursts of muscle sympathetic nerve activity per minute (MSNA bursts/min) (mean ± standard deviation).

Participant initials	**Observer one (MSNA bursts/min) average**	**Observer two (MSNA bursts/min) average**	**Coefficient of Variation (%)**
KP	42±1	46±1	5
AM	60±1	60±1	0
PR	35±2	38±1	5
GS	32±3	32±2	6
Average	**42±13**	**44±12**	**4±3**

Table 2-9 Repeat interobserver analysis of bursts muscle sympathetic nerve activity per 100 heart beats (100Hb) (MSNA bursts/100Hb) (mean ± standard deviation).

Participant initials	**Observer one (MSNA bursts/100Hb) average**	**Observer two (MSNA bursts/100Hb) average**	**Coefficient of Variation (%)**
KP	79±1	85±2	4
AM	71±1	76±2	4
PR	76±2	81±0.2	4
GS	54±3	55±3	5
Average	**70±11**	**74±13**	**4±1**

2.11 Figures

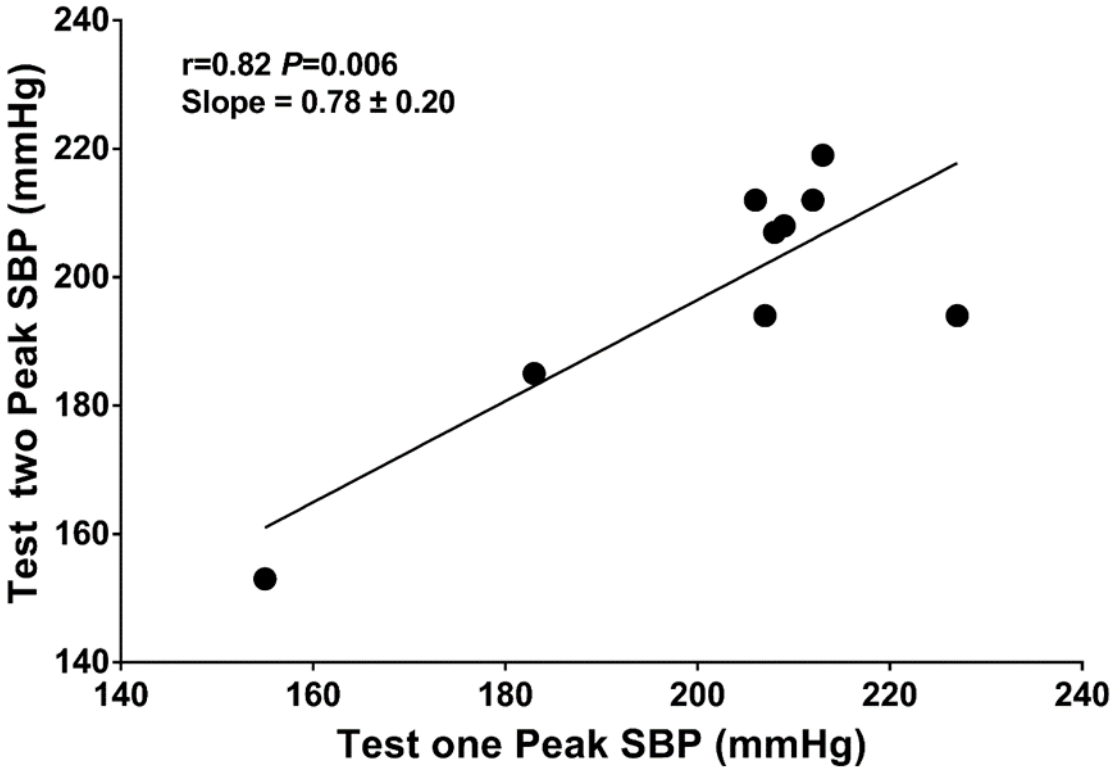

Figure 2-1 Repeatability of systolic blood pressure (SBP) during incremental cycle ergometer exercise test ($\dot{V}O_2$ peak test) on two separate days.
Linear regression of peak SBP assessment during an $\dot{V}O_2$ peak test on two separate days, without an intervention (n = 9). There was a positive correlation between the peak SBP measured on two separate days (Pearson's r = 0.82, P=0.006).

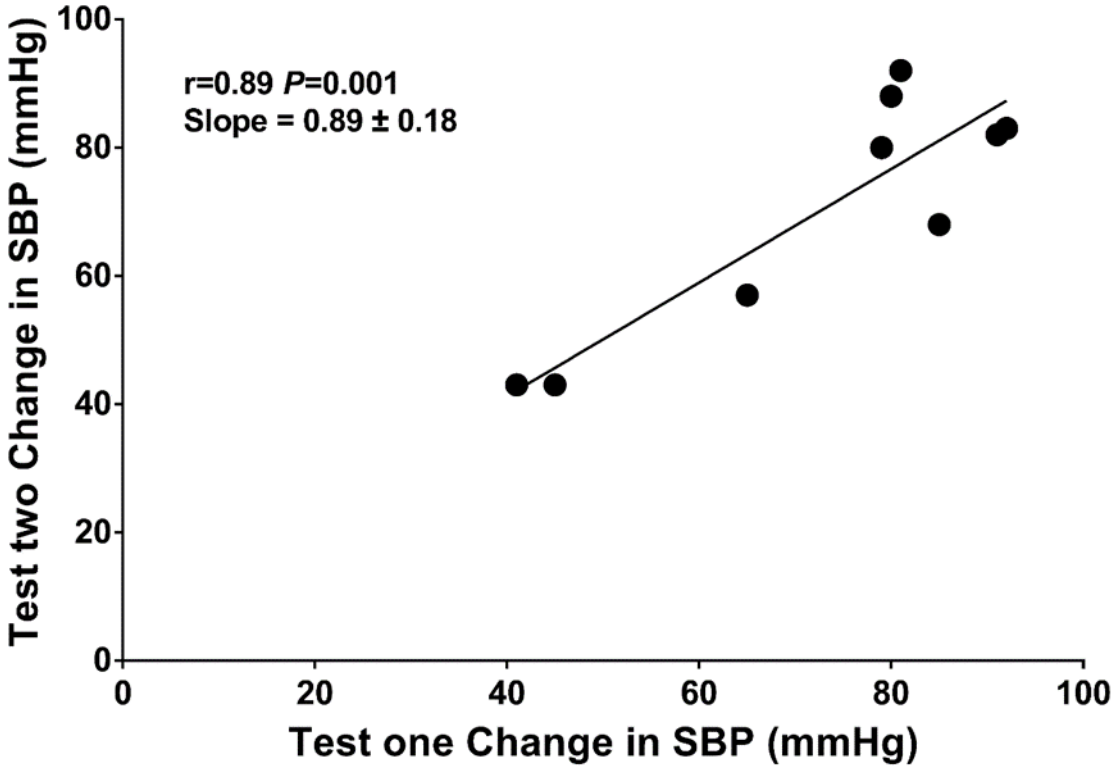

Figure 2-2 Repeatability of the change in systolic blood pressure (SBP) during incremental cycle ergometer exercise test ($\dot{V}O2$ peak test) on two separate days. Linear regression of change in absolute peak SBP assessment during an $\dot{V}O2$ peak test on two separate days without an intervention (n = 9). There was a positive correlation between the peak SBP measured on two separate days (Pearson's r = 0.89, *P=0.001).*

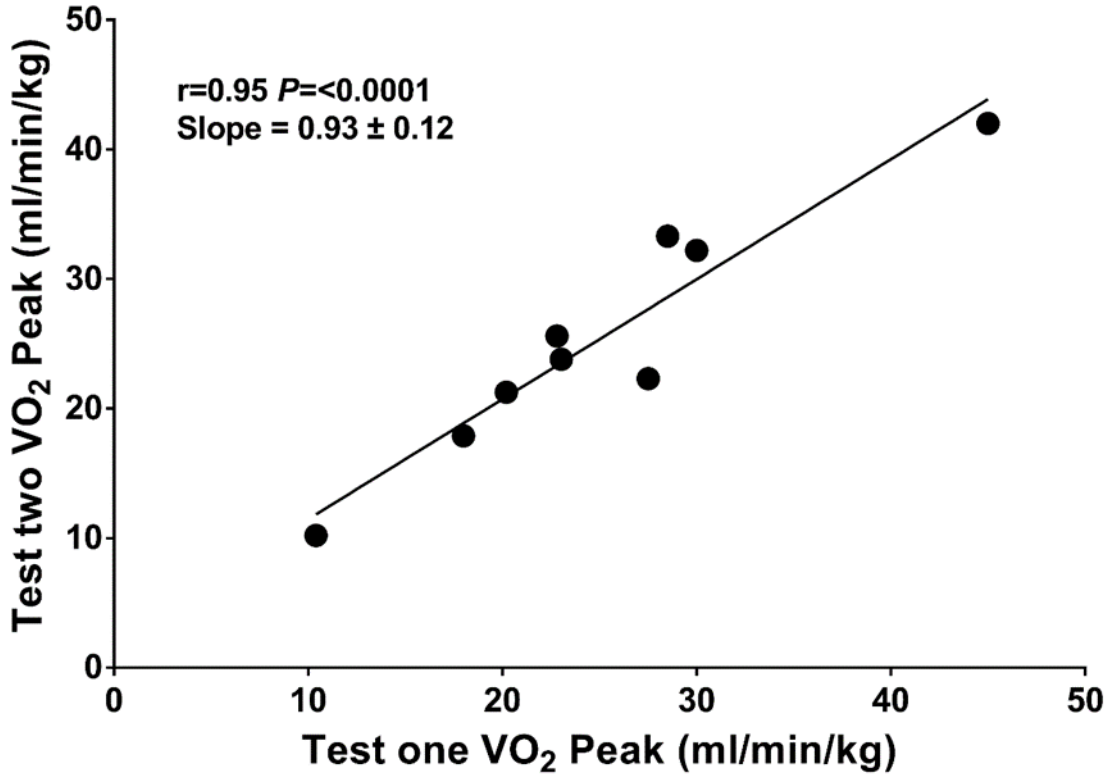

Figure 2-3 Repeatability of *V̇*O2 peak scores (ml/min/kg) during incremental cycle ergometer exercise test (*V̇*O2 peak test) on two separate days without intervention. Linear regression of *V̇*O2 peak scores (ml/min/kg) measured from an incremental cycle ergometer exercise test (*V̇*O2 peak test) on two separate days without an intervention (n = 9). There was a positive correlation between the peak SBP measured on two separate days (Pearson's r = 0.95, *P=<0.0001).*

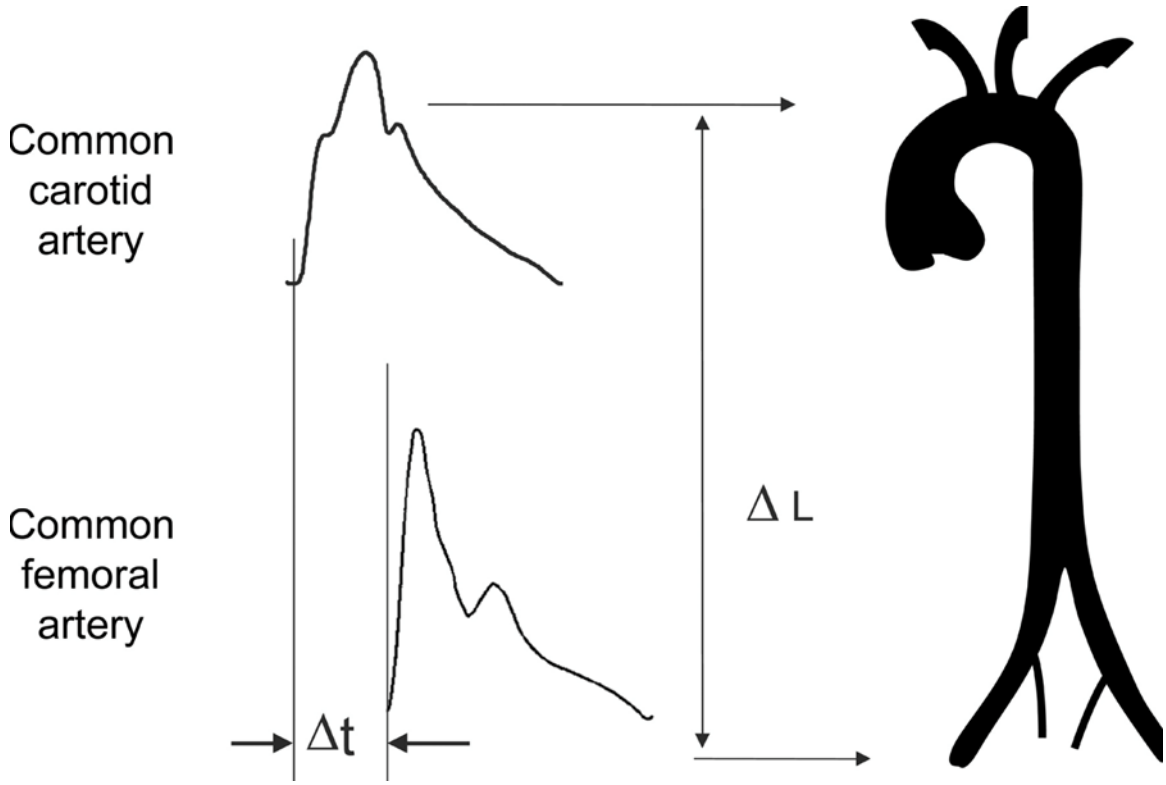

Figure 2-4 A schematic of the measurement of pulse wave velocity. Measurement of pulse wave velocity using the SphygmoCor System (AtCor Medical, Sydney SpA). Pulse transmit times are calculated from the R wave of the electrocardiogram (ECG) to the onset of the pulse pressure wave. The difference in the pulse transmit time is then calculated for the 2 sites being assessed. Further, the difference in the distance between the two sites and the suprasternal notch is then calculated. To assess pulse wave velocity (PWV) the difference in the pulse transmission time is divided by the distance between the carotid and femoral pulse (image from Laurent et al. (2006)).

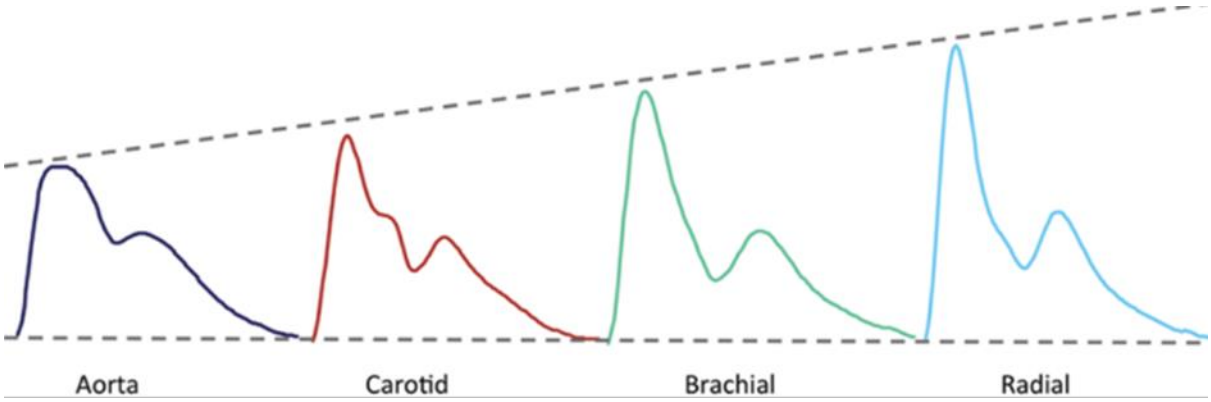

Figure 2-5 Systolic blood pressure amplification from the aorta to the radial artery. Two models have been described to explain this systolic blood pressure augmentation, wave reflection model and the adapted Windkessel approach.

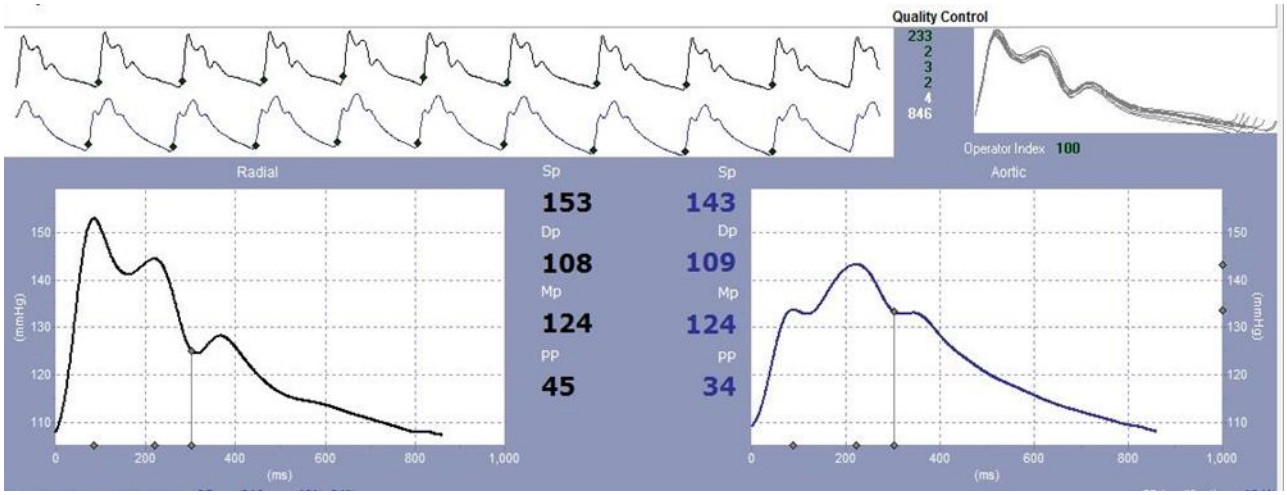

Figure 2-6 Pulse wave analysis in a patient with untreated hypertension.

A peripheral arterial waveform from the radial pulse (left), SBP (Sp), DBP (Dp), MAP (Mp) and pulse pressure (PP) are all calculated automatically from the SpygmoCor system. On the right is a reconstructed aortic pressure waveform from the radial pulse via a transfer function.

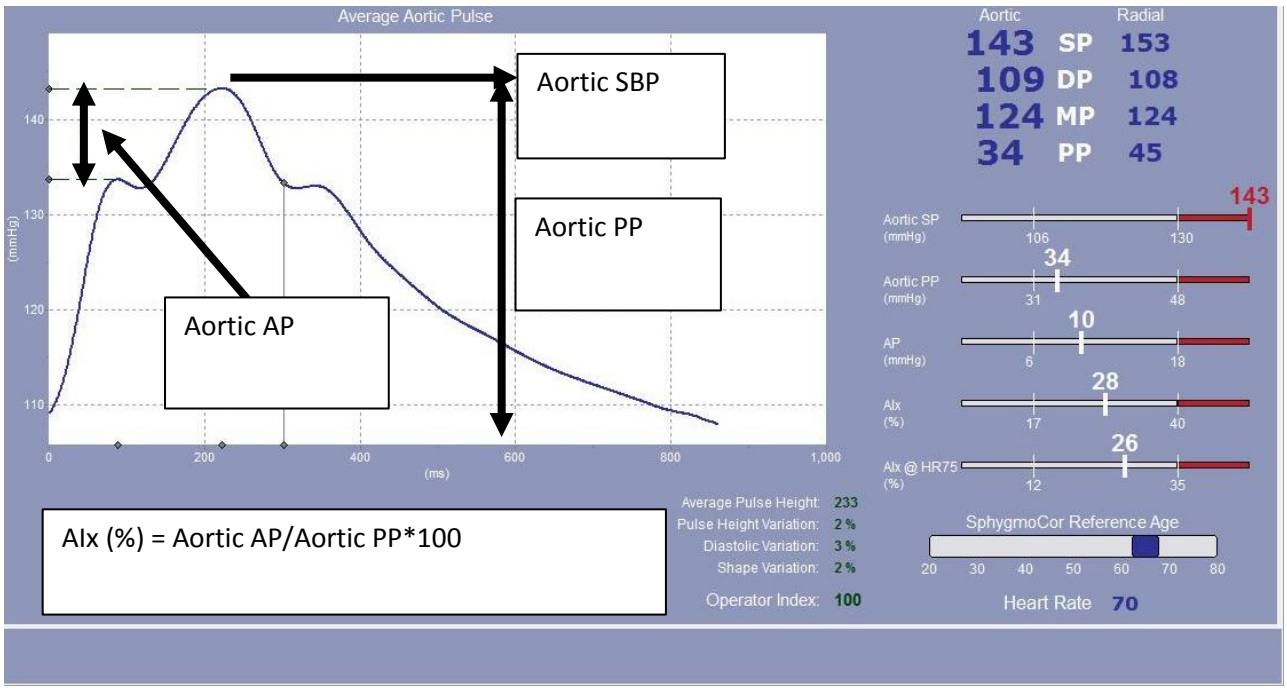

Figure 2-7 An aortic pressure waveform from an untreated hypertensive human. AP = aortic augmentation pressure, Aortic SBP = Aortic SBP and Aortic PP = Aortic Pulse Pressure. AIx (%) is the augmentation index and is calculated from Aortic Arterial Pressure/Aortic Pulse pressure*100.

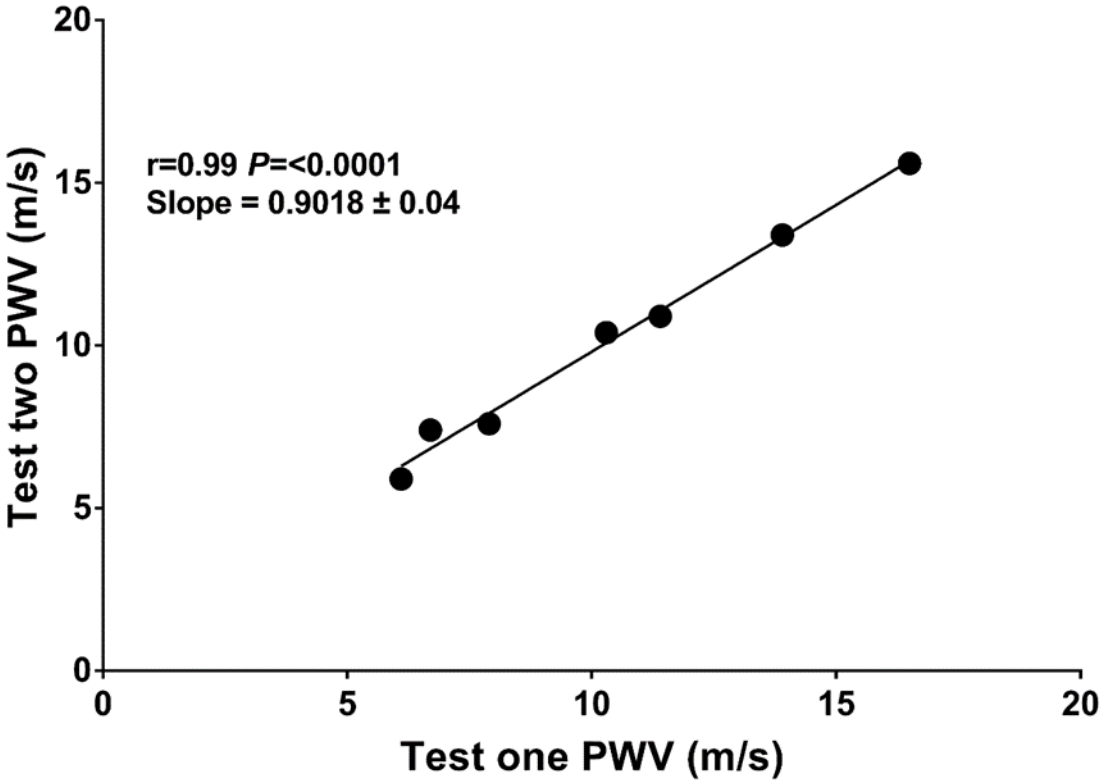

Figure 2-8 Repeatability of pulse wave velocity (m/s) (PWV) at rest.

Linear regression of PWV measured from the carotid-femoral pulse on two separate days without an intervention (n = 7). There was a positive correlation between the PWV (carotid-femoral) measured on two separate days (Pearson's r = 0.99, *P=<0.0001).*

Figure 2-9 An illustration of the experimental set up used for the Finometer Pro system (Finometer, FMS, Netherlands).

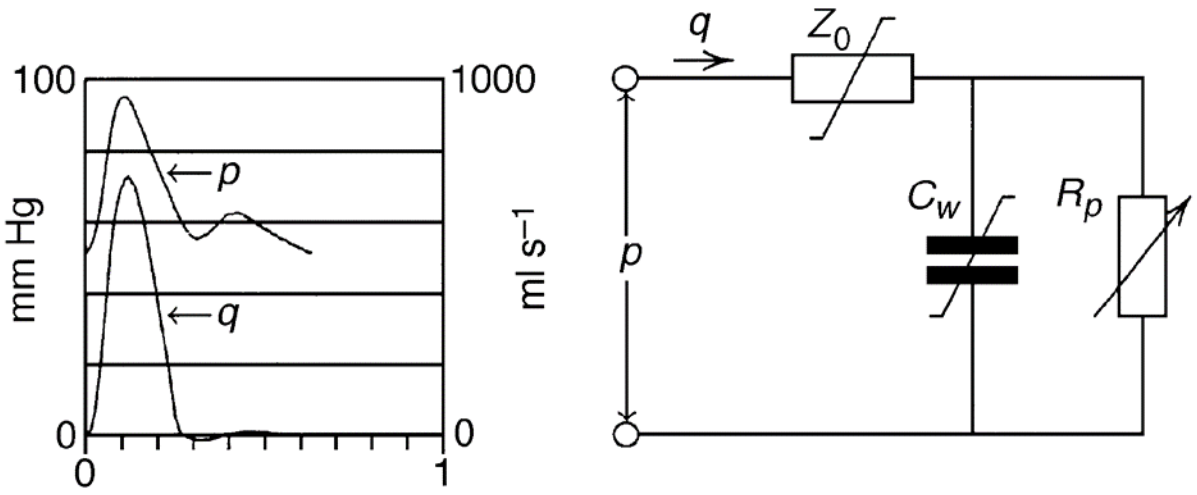

Figure 2-10 Schematic illustration of the 3-element model used for model flow. The left side shows the simulated aortic flow pulse and the right side shows the self-adapting 3- element model. Arterial pressure (*p*) is inputted into the model. Z_0, characteristic impedance of the aorta; C_W, Windkessel compliance' R_P, total peripheral resistance. The arrow through R_P shows that it changes during changes in arterial pressure and the line through C_W and Z_0 indicates a non-linear relationship with changes in pressure. This model is simulated beat-to-beat and produces a flow curve (left side) labelled q. The area under the curve (q) is taken as the stroke volume. Image taken from Jansen et al. (2001).

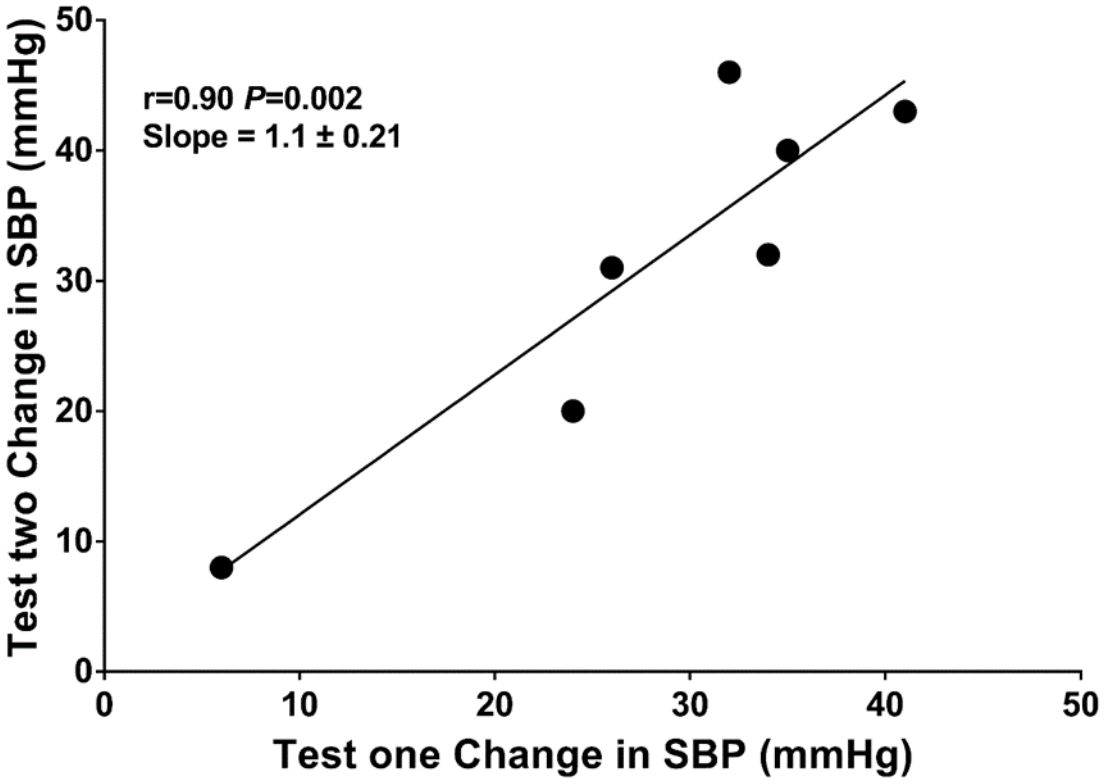

Figure 2-11 Correlation of the change in systolic blood pressure (SBP) from a baseline period during post-exercise ischemia (PEI) on two separate days.

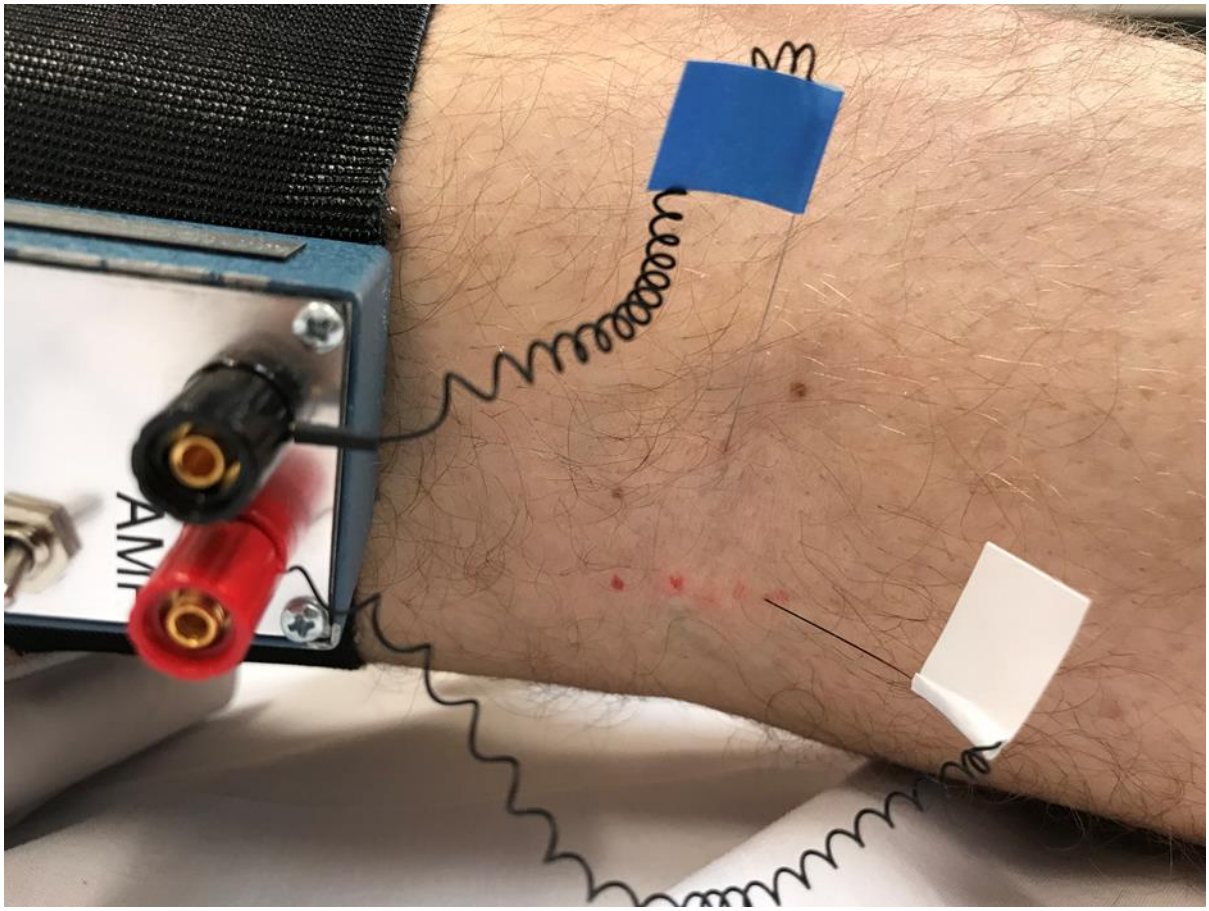

Figure 2-12 Set up for peroneal nerve microneurography in the right leg. Cutaneous electrical stimulation was used to locate the position of the peroneal nerve. When the electrical stimulation led to dorsiflexion a small red dot was marked. As this is an indicator of the position of the deep peroneal nerve. A 35 mm tungsten reference electrode (blue flag) was then inserted into the participants skin around 2-3 cm away from the deep peroneal nerve. A 35 mm active tungsten electrode (white flag) was then inserted through the skin into the peroneal nerve. The active and reference electrode were attached to a pre-amplifier on the left of the image attached just above the knee.

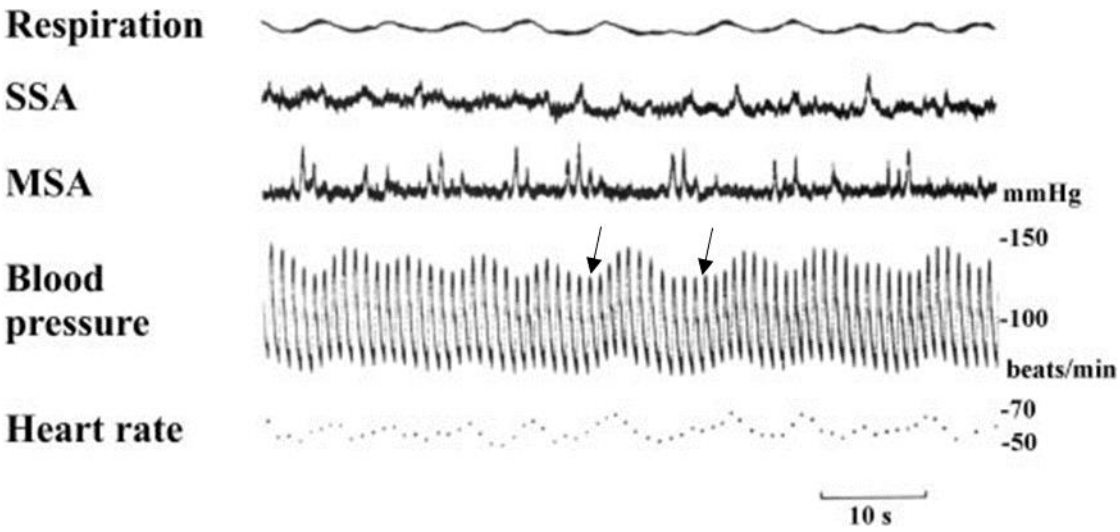

Figure 2-13 Resting muscle (MSA) and skin (SSA) sympathetic nerve activity. The example MSA and SSA shown here are integrated neural outputs from a raw trace, time constant, 0.1s). It is clearly demonstrated that when BP dips (black arrows) it leads to increases in MSA (cardiac rhythmicity) but not changes in SSA. (Image from Delius et al., 1972a, Delius et al., 1972b).

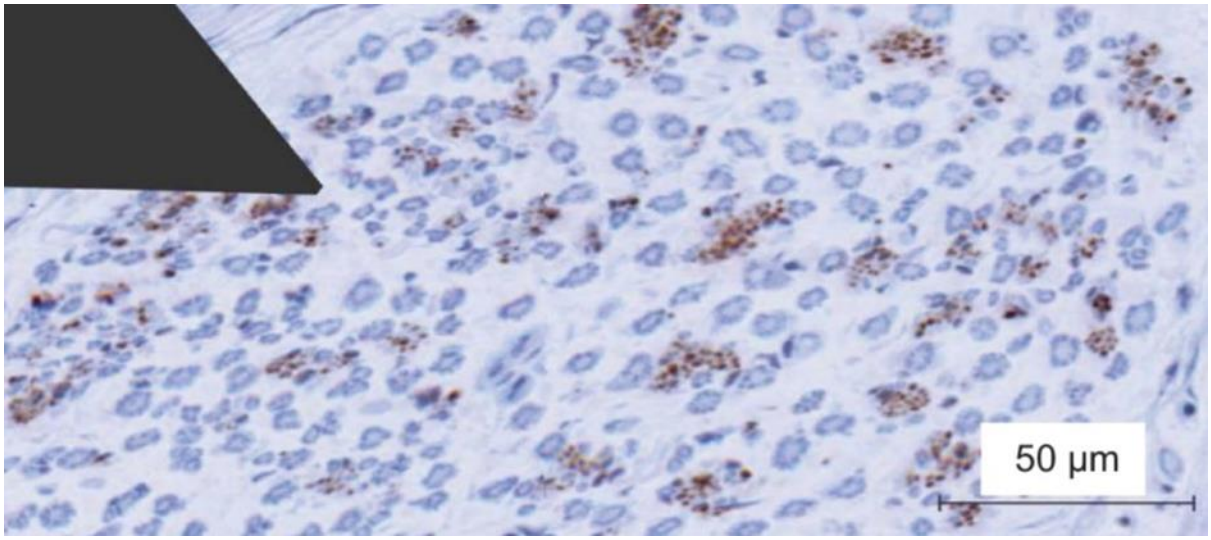

Figure 2-14 The common peroneal nerves surgically excised from a human cadaver.

Tyrosine hydroxylase containing axons were assessed in the common peroneal nerve. This figure shows a magnification of a tungsten needle (top left) and of one fascicle within the common peroneal nerve, the brown staining indicates tyrosine hydroxylase containing sympathetic nerve fibres. Some of these fascicles contain no sympathetic nerve fibres. Image from Tompkins et al. (2013).

Chapter 3 The Exercise Pressor Reflex in Humans with Hypertension

Part of this study has been published in *Hypertension* (see appendix 2, page 377) (Chant et al., 2018).

3.1 Introduction

It is well established that a sedentary lifestyle is a key risk factor for the development of hypertension, and that chronic aerobic endurance training can lower resting blood pressure (BP) and mortality rates in these individuals (Whelton et al., 2002, Rossi et al., 2012). However, during an acute bout of dynamic or isometric exercise, individuals with untreated hypertension have an exaggerated increase in BP compared to normotensive controls (Delaney et al., 2010, Greaney et al., 2015a, Greaney et al., 2014, Aoki et al., 1983, Choi et al., 2013, Brorson et al., 1978, Seguro et al., 1991, Barbosa et al., 2016). Worryingly, an exaggerated increase in BP during an acute bout of dynamic exercise is an independent risk for adverse cardiovascular (CV) events (Kjeldsen et al., 1997, Kjeldsen et al., 2001) any type of stroke (Kurl et al., 2001, Laukkanen et al., 2006), left-ventricular hypertrophy (Ren et al., 1985, Papademetriou et al., 1989), myocardial infarction (Laukkanen et al., 2006, Kjeldsen et al., 1997, Kjeldsen et al., 2001, Mundal et al., 1996, Filipovsky et al., 1992, Kohl et al., 1996) and total mortality (Filipovsky et al., 1992, Kohl et al., 1996, Fagard et al., 1996) independent of resting BP.

Adjustments in the autonomic nervous system during exercise are mediated by central command (feed-forward signals from higher brain centres) (Zuntz and Geppert, 1886, Goodwin et al., 1972), mechanically sensitive afferents (group III) and metabolically sensitive afferents (group IV) from the skeletal muscle

(collectively known as the exercise pressor reflex) (Alam and Smirk, 1937, Mense and Stahnke, 1983, Kaufman et al., 1983). In addition, a proportion of the group III afferents are polymodal and are stimulated by metabolic stimuli (Kaufman et al., 1983). The exercise pressor reflex is critical in regulating the normal CV response to exercise, including the maintenance of perfusion pressure to the active skeletal muscle (Amann et al., 2011a, Amann et al., 2010, Amann and Dempsey, 2008, Amann et al., 2011b). Individuals with untreated hypertension have a higher level of sympathetic nerve activity (SNA) at rest (Warnert et al., 2016) and an exaggerated rise in SNA during dynamic (Sausen et al., 2009, Vongpatanasin et al., 2011) and isometric exercise (Delaney et al., 2010) when compared to age-matched normotensive individuals. An intrathecal opioid agonist (fentanyl), which inhibits the exercise pressor reflex at the dorsal horn, normalised the exaggerated BP rise observed in patients with untreated hypertension compared to healthy age-matched individuals (Barbosa et al., 2016). This study highlights the importance of the exercise pressor reflex in mediating the abnormal CV response to exercise in hypertension. In animal models of hypertension, activation of the metaboreflex leads to exaggerated increases in mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) when compared to normotensive animals (Mizuno et al., 2014b, Mizuno et al., 2013). Additionally, work in humans has also highlighted a key role of the metaboreflex (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014) in driving an abnormal elevation in MSNA and BP during exercise in patients with untreated hypertension.

Currently, our understanding of the BP response to exercise and isolation of the metaboreflex in patients with treated hypertension is limited. Research in human hypertension has focused on patients with untreated hypertension or withdrawn from their medication 48-hours prior to participation. It is important to establish whether individuals with untreated hypertension who have their BP controlled by anti-hypertensive medication still have an exaggerated BP response to metaboreflex isolation. Exaggerated BP responses to exercise in patients with hypertension are associated with blunted reductions in left ventricular hypertrophy during anti-hypertensive therapy (Mizuno et al., 2016b). Worryingly, despite adequate BP control, patients with treated-controlled hypertension have an increased risk of stroke, CV disease and total mortality when compared to normotensive individuals (Lawlor et al., 2011, Brown et al., 2013, Almgren et al., 2005). Although the exact mechanism for this is unknown, first-line treatment for hypertension (including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers and diuretics) does not antagonise any of the known receptors that sensitize the metaboreflex in hypertension (acid sensing ion channels (ASIC3), purinergic receptors (P2X2/3 and, P2X3 subtypes) and transient receptor potential cation channel subfamily V member 1 (TRPv1) (Light et al., 2008, Pollak et al., 2014, Stone et al., 2015). The aim of this study was to assess whether patients with treated-controlled, treated-uncontrolled and untreated hypertension will have an excessive rise in BP during 1) an incremental exercise test to peak oxygen consumption ($\dot{V}O_2$ peak test) and 2) isolation of the metaboreflex (post-exercise ischemia; PEI). Based on previous research, the hypothesis was that there would be a difference in the BP response to incremental cycle ergometer exercise and metaboreflex isolation between

normotensive controls versus treated-controlled, treated-uncontrolled and untreated patients with hypertension.

3.2 Methods

3.2.1 Participants

69 participants were screened for this study and 10 were excluded due to screening failure (Figure 3.1, page 196). 16 normotensives, 16 treated-controlled, 16 treated-uncontrolled and 11 untreated hypertensives (N=59) were matched for age, body mass index (BMI), and for CV fitness ($\dot{V}O_2$ peak) (Table 3.1, page 190). Ethical approval for this study was granted by the Southwest-Exeter NHS REC (16/SW/0004). Volunteers were recruited from our groups' specialist hypertension clinic at University Hospitals Bristol Trust and Foundation, Bristol. The remaining participants with hypertension were recruited from the surrounding area. Normotensive control participants were recruited from our existing database of willing participants and from advertising locally. Participants gave informed consent during an initial screening visit prior to participation. Table 3.1 (page 190) shows participant demographics. Overall, 29 females (49%) were recruited to this study and 27 of which were postmenopausal (93%). The remaining 7% were perimenopausal. The study was conducted at the Clinical Research and Imaging Centre (CRiC), Bristol. All participants attended the CRiC Bristol at a similar time of day and all lab conditions were controlled to a set temperature (22°C). Participants were asked to refrain from alcohol and caffeine for 12 hours before the study visits. In addition, participants were asked to abstain from high-intensity exercise for at least 24-hours prior to participation. Participants in the treated-

hypertensive group were asked to take their anti-hypertensive medication as normal.

In accordance with the National Institute for Health and Care Excellence (NICE) guidelines (NICE, 2011), normotension was defined as clinic BP <140/90 mmHg and daytime ambulatory blood pressure monitoring (ABPM) =<135/85 mmHg and free from anti-hypertensive medication(s). Untreated hypertension was defined as daytime ABPM =>135/85mmHg and not currently taking any anti-hypertensive medication (s). Treated-controlled hypertension was defined as daytime ABPM (<135/85 mmHg) and the use of one or more anti-hypertensive medications. Treated-uncontrolled hypertension was defined as poor BP control on daytime ABPM (=>135/85 mmHg) whilst taking one or more anti-hypertensive medications. Exclusion criteria included; diabetes mellitus (urine dipstick test and self-reported), BMI (>30 kg/m^2), pregnancy, major illness (such as cancer), overt respiratory-CV disease (other than hypertension), $\dot{V}O_2$ peak > 40 ml/min/kg and febrile illness with 2 weeks of the study. Participants were excluded if $\dot{V}O_2$ peak > 40 ml/min/kg because the BP response to exercise may be modulated by CV fitness levels (Kokkinos, 2014). In a group of pre-hypertensive hypertensive individuals (average age 52±10), high-fit individuals had a reduced BP during moderate and maximal treadmill exercise when compared to unfit individuals (Kokkinos et al., 2007). Furthermore, for the average age of the participants in this study a $\dot{V}O_2$ peak > 40 ml/min/kg is extreme and way above the average for both males (29.4±7.9 ml/min/kg) and females (20.7±5 ml/min/kg) (Kaminsky et al., 2015). Participants were excluded from the study if $\dot{V}O_2$ peak > 40 ml/min/kg following the screening visit. Participants were also asked to avoid the use of

painkillers such as aspirin, paracetamol or anti-inflammatory drugs (e.g., ibuprofen) for 24 hours prior to the study visits. Participants were asked to refrain from these medications due to their known inhibitory effects on exercise BP (Drew et al., 2013, Cui et al., 2007, Cui et al., 2008b).

3.2.2 Study Design

This was a case-control study. The researcher was blinded to the data analysis until all data was analysed.

3.2.3 Screening procedures

Participants attended an initial screening visit where resting clinic BP was measured using an automated cuff (Omron, The Netherlands). In line with the European Society of Hypertension (O'Brien et al., 2000, O'Brien et al., 2001, O'Brien et al., 2013), the first BP measurement was ignored which was followed by a BP reading on both the left and right arm and a final reading was then taken on the arm where BP was highest. A research nurse then fitted participants with a 12-lead electrocardiogram (ECG) prior to a maximal exercise test ($\dot{V}O_2$ peak test) on a cycle ergometer to rule out any CV abnormalities. The ECG was checked by a cardiologist. Participants were fitted with a 24-hour ABPM to classify participants to their respected groups (Spacelabs, OSI Systems Company, USA). The 24-hour ABPM assessed BP every 30 minutes during the daytime and once per hour throughout sleeping hours. Participants also completed a 24-hour BP diary. The ABPM was delayed for 24-hours following the $\dot{V}O_2$ peak to avoid post-

exercise hypotension (Brito et al., 2014). A flow diagram for the initial screening can be seen below (Figure 3.2, page 197).

3.2.3.1 V̇O2 peak assessment

$\dot{V}O_2$ peak was assessed using a 25 watts/min ramp protocol on a cycle ergometer, which started at 0 watts (Love Medical, Manchester, United Kingdom). The test began with a 5-minute baseline period which was used to compare all haemodynamic variables measured during the $\dot{V}O_2$ peak test. Participants were instructed to maintain a cadence during the test of between 60-80 revolutions per minute. $\dot{V}O_2$ peak (mL/min/kg) was defined as the mean value of $\dot{V}O_2$ attained during the final 30 seconds of exercise (Wylie et al., 2016). $\dot{V}O_2$ peak was defined by the following criteria: an respiratory exchange ratio (RER) of >1.15 (Issekutz et al., 1962), HR of 85% maximum (220-age) (Brown et al., 2002) and a rating of perceived exertion of > 17 on the 20 point Borg Scale (rating perceived exertion) (Church et al., 2008). For more detailed information see Chapter 2 (section 2.3.1, page 94).

3.2.4 Study Visit design

Participants returned at a similar time of day for a follow up visit to the screening visit with at least 48 hours between. The study day involved the assessment of clinic BP using the same protocol from the initial screening visit. The maximal voluntary contraction (MVC) was then assessed using a handgrip dynamometer on the dominant hand. To assess the MVC, participants performed a maximal contraction of the handgrip dynamometer three times with at least 30 seconds

between each attempt. The highest value of the three attempts was regarded as the MVC (Delaney et al., 2010). An outline of the study visit design can be seen in Figure 3.3 (page 198).

3.2.4.1 Handgrip and Metaboreflex testing

Prior to handgrip testing the participant was asked to relax for 10 minutes before the onset of a baseline period of 10 minutes. Following the baseline assessment, the participant performed 1 minute of isometric handgrip exercise at 30% MVC (Figure 3.4, page 199). An occlusion cuff was inflated to suprasystolic pressure (240 mmHg) over the brachial artery on the exercising arm at 1 minute and remained inflated for a further 1 minute 30 seconds and was regarded as PEI (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014, Alam and Smirk, 1937) (Figure 3.4, page 199). PEI represents isolation of the metaboreflex, independent of the mechanoreflex and central command (Kaufman et al., 1983, Kaufman et al., 1984). The change in systolic blood pressure (SBP) from baseline during the final minute of the PEI period was used as an assessment of the sensitivity of the metaboreflex (Figure 3.4, page 199).

3.2.5 Physiological Monitoring During $\dot{V}O_2$ Peak and Metaboreflex Testing

3.2.5.1 $\dot{V}O_2$ peak test

BP was measured every 1.5 minutes during the $\dot{V}O_2$ peak testing on the left arm of the participant using an automated sphygmomanometer which had not been validated for use during exercise (Love Medical, Manchester, UK). To measure

HR a 12-lead ECG (Love Medical, Manchester, UK) was used during the $\dot{V}O_2$ peak test. Breathing frequency, tidal volume, minute ventilation (breathing frequency * tidal volume), $\dot{V}O_2$ and volume of carbon dioxide expired ($\dot{V}ECO_2$) were assessed using an Ergoflow flow sensor for spirometry (Ergostik CPET system, Love Medical, Manchester, UK). For more detailed information see Chapter 2 (section 2.3.1, page 94).

3.2.5.2 Metaboreflex testing

During isometric handgrip exercise and PEI, the change in BP was assessed on a beat-to-beat basis using finger plethysmography (Finometer; Finapres Medical Systems, The Netherlands). The change in HR was measured using a 3-lead ECG. The change in BP and HR were compared to a baseline period of 10 minutes. Data was collected during baseline, isometric handgrip exercise and PEI using a data acquisition system (LabChart 7; AD Instruments).

3.2.6 Power calculations

To find a significant difference (P=<0.05) in peak SBP during maximal exercise testing ($\dot{V}$O2 peak test), 60 participants will provide the power >80%. A previous study (Mizuno et al., 2016b) found that an exaggerated SBP during exercise testing was 214±12 mm Hg (n=40) and a normal SBP response to exercise was 172±8 mm Hg (n=63). A large effect size was calculated from these results (f = 2.04). It was assumed that there would be more variance in our data because this previous study grouped participants into subgroups based on their SBP response to exercise (e.g. low-medium-high) and was therefore left with a small standard

deviation and very large effect size, therefore a smaller effect size was used for this study (d = 0.45).

3.2.7 Data analysis

3.2.7.1 $\dot{V}O_2$ peak test

The previous literature that has assessed BP responses to maximal exercise testing ($\dot{V}O_2$ peak testing) has focused on peak BP. It is currently unclear at what % of $\dot{V}O_2$ peak testing the exaggerated BP rise occurs in patients with hypertension (Kjeldsen et al., 2001, Kjeldsen et al., 1997, Mundal et al., 1996, Kurl et al., 2001, Fagard et al., 1996). Therefore, to assess at what % of $\dot{V}O_2$ peak testing that the rise in BP is different between normotensives, untreated hypertensives, treated-controlled hypertensives and treated-uncontrolled hypertensives two methods of analysis were used:

1. The absolute change (Δ), percentage change (%) and absolute BP during the $\dot{V}O_2$ peak test was assessed in five different epochs of: 0-25, 26-50, 51-75, 76-100% and the peak BP attained during the $\dot{V}O_2$ peak test.
2. The peak absolute change in SBP attained during the test was divided by the peak exercise time to calculate the rise in SBP per minute (SBP/min).

This analysis was completed during $\dot{V}O_2$ peak testing for SBP, diastolic blood pressure (DBP), MAP, pulse pressure (PP), HR, tidal volume, breathing frequency and minute ventilation. The anaerobic threshold was calculated using the V-slope method, briefly, a moving average of V_ECO_2 (L/min) was plotted against a moving average of $\dot{V}O_2$ (L/min), the intercept at which V_ECO_2 (L/min) crosses $\dot{V}O_2$ (L/min) was regarded at the anaerobic threshold (Svedahl and

MacIntosh, 2003, Sue et al., 1988, Schneider et al., 1993). The anaerobic threshold is reported as a percentage value of $\dot{V}O_2$ peak. Additionally, the ventilatory efficiency slopes (VE/V_ECO_2 slope), defined as the relationship between minute ventilation and carbon dioxide production were assessed (Rausch et al., 2013). A VE/V_ECO_2 slope >34 has been shown to highlight high risk pulmonary hypertension and heart failure patients (Rausch et al., 2013, Bard et al., 2006). However, little is known about the VE/V_ECO_2 slope in patients with essential hypertension, treated or untreated. The VE/V_ECO_2 slope is assessed by plotting VE against V_ECO_2 from the onset of exercise to peak exercise or baseline to the anaerobic threshold (Bard et al., 2006). The VE/V_ECO_2 slope has increased prognostic value when measured from the onset of exercise to peak exercise in heart failure (Bard et al., 2006).

3.2.7.2 Metaboreflex testing

The absolute change in SBP, DBP, MAP, PP and HR during isometric handgrip exercise and PEI were measured over 30 second epochs (Delaney et al., 2010). The change in SBP, DBP, MAP, PP and HR were assessed by comparing to a 10-minute baseline prior to the onset of isometric handgrip exercise. PEI was split into 3 epochs, PEI 1, PEI 2 and PEI 3 which accounted for the initial drop in BP following the withdrawal of isometric handgrip exercise expected in PEI 1 (Delaney et al., 2010). A 5-minute recovery period following metaboreflex testing was split into 5 1-minute epochs (R1, R2, R3, R4, R5). The analysis was completed using LabChart 7 (AD Instruments), Spike 2 (Cambridge Electronic Designs) and Microsoft Excel (Microsoft Corp., Redmond, WA).

3.2.8 Additional physiological measurements

3.2.8.1 Heart Rate Variability

Heart rate variability (HRV) (LabChart 8; AD Instruments) was calculated using the Lomb Periodogram nonparametric method for spectral analysis from the 10 minute baseline period and the 5 minute recovery period following PEI (Krafty et al., 2014). The software used for analysis (LabChart 7, AD Instruments) automatically removes any abnormal R-R intervals for analysis (e.g. ectopics). The following parameters were used for the HRV analysis: low-frequency (LF) power = 0.04-0.15 Hz and high-frequency (HF) power =0.15-0.40 Hz (Billman, 2013). HF represents a parasympathetic component related to HR changes mediated by respiration and LF represents baroreceptor modulation (Miranda Dantas et al., 2012, Akselrod et al., 1981, Pomeranz et al., 1985). Patients with baroreflex failure have a reduced LF power during tyramine administration when compared to individuals with a normally functioning baroreflex (Moak et al., 2007). The European Society of Cardiology guidelines for HRV state that at least 60 seconds of ECG recording is needed for LF and 120 seconds for HF, therefore HRV analysis was analysed at rest and recovery but not during isometric handgrip or PEI (Force, 1996). The very low-frequency (VLF) (0.0033 – 0.04 Hz) power for this study is not reported as longer time periods (24-hours) are needed for analysis of this component of HRV (Kleiger et al., 2005, Force, 1996). For HRV, the LF/HF ratio, the LF and HF are reported as normalised to total power (HF nu and LF nu) and as total power (ms^2).

In addition, HRV was assessed using the time-domain method using LabChart version 8 (AD Instruments). The following parameters are reported from the time-domain analysis. Firstly, the standard deviation of the RR interval (SDRR). As the SDRR is equal mathematically to the total power of the spectral analysis, the SDRR is a reflection of the combination of the cyclic components of the variability from the recording period (Force, 1996). The ESC guidelines suggest that a minimum period of 5 minutes should be used to assess SDRR (Force, 1996). However, with longer periods of analysis (e.g. 24 hours) the variability of the RR interval is larger and when using shorter analysis time lengths (e.g. 5 minutes) the SDRR may only represent the high frequency component of HRV. Other methods, such as the standard deviation of the average RR interval (SDARR) and standard deviation of the RR interval require longer periods of recording for accurate assessment (e.g. 24 hours). In addition, the square root of the mean standard differences (RMSSD) of successive RR intervals and the pRR50 represent the high frequency component of the HRV (Force, 1996, Hartwich et al., 2013, Hartwich et al., 2011) and can be assessed accurately over short periods of time (e.g. 5 minutes) (Force, 1996). The pRR50 is the RR50, the amount of RR intervals above 50 ms divided by the total number of RR intervals (Force, 1996).

3.2.8.2 Spontaneous Cardiac Baroreflex Sensitivity

The spontaneous cardiac baroreflex sensitivity was assessed using the sequence method in all of the participants from the 10-minute baseline period prior to metaboreflex testing and during the 5-minute recovery period. The up and

down sequences of SBP and the R-R interval were used for this analysis (Parati et al., 1988). More specifically, up sequences require at least 3 or more cardiac cycles where there is an increase in SBP and the R-R interval (Parati et al., 1988). Similarly, down sequences require at least 3 or more cardiac cycles where there is a decrease in SBP and the R-R interval (Parati et al., 1988). Any points at which one parameter was increasing and the other decreasing were not included in the analysis (Parlow et al., 1995). The thresholds for cardiac baroreflex sensitivity are a minimum change in SBP of 1 mmHg and a change in the R-R interval of 6 msec (Parati et al., 1988, Taylor et al., 2015). The change in R-R interval to a given change in SBP was chosen as there is evidence that this related to vagal tone at the level of the heart (Parker et al., 1984). The sensitivity of the cardiac baroreflex was assessed by plotting the change in the R-R interval against the absolute change in the SBP, the accepted r value was set at =>0.80 (Parati et al., 1988). A minimum number of 3 sequences was required to perform analysis of the cardiac baroreflex sensitivity (Parati et al., 1988). LabChart 7 (AD Instruments), Spike 2 (Cambridge Electronic Designs), Microsoft Excel (Microsoft Corp., Redmond, WA) and CardioSeries v2.4 (http://www.danielpenteado.com) were used to assess cardiac baroreflex sensitivity. An issue with the sequencing method is that over short periods of time increases in SBP are not always coupled to changes in the R-R interval (Di Rienzo et al., 2001). Whereas over a 24-hour period typically 80 sequences are found per hour in healthy individuals (Di Rienzo et al., 2001, Parati et al., 1988). The baroreflex effectiveness index was also assessed which uses the same 3 or more cardiac cycles where there is an increase in SBP and the R-R interval (Di Rienzo et al., 2001). In addition, this method also uses a minimum change in SBP of 1 mmHg and a change in the R-

R interval of 6 msec (Di Rienzo et al., 2001). Unlike the cardiac baroreflex sensitivity analysis the baroreflex effectiveness index considers SBP and R-R interval ramps from 0, 1 and 2 beat lags. This inclusion criteria were based on previous research that found SBP and R-R interval ramps with 0,1 and 2 beat delays are under baroreflex control (Blaber et al., 1995). The baroreflex efficiency index was calculated as the total number of SBP ramps divided by the number of R-R interval/SBP sequences (Di Rienzo et al., 2001). The average of the baroreflex efficiency index was taken from 0,1 and 2 beat delays to give an overall baroreflex efficiency index (Di Rienzo et al., 2001).

3.2.9 Statistical Analysis

Baseline characteristics were compared using an ordinary 1-way analysis of variance (ANOVA) with a Tukey test for multiple comparisons if a significant interaction effect was found. Data were tested for normal distribution and homogeneity of variance using a D'Agostino-Pearson omnibus K2 normality test and Levine's test for homogeneity of variances respectively. The α level was set at 0.05.

The group averages (normotensives, untreated hypertensives, treated-controlled hypertensives and treated-uncontrolled hypertensives) for the absolute change (Δ), percentage change (%) and absolute SBP, DBP, MAP, PP, HR, tidal volume, breathing frequency and minute ventilation during the $\dot{V}O_2$ peak test were compared using an ordinary 2-way ANOVA. A Tukey test for multiple comparisons was used if a significant interaction effect was found with the

ordinary 2-way ANOVA. The group averages for the absolute change (Δ) in SBP, DBP, MAP, PP and HR during isometric handgrip and PEI were compared using an ordinary 2-way ANOVA. A Tukey test for multiple comparisons was used if a significant interaction effect was found with the ordinary 2-way ANOVA. A Pearson's correlation coefficient was performed to assess whether the was a relationship between the change in absolute SBP during PEI and the absolute change in SBP at peak exercise during the $\dot{V}O_2$ peak test. All data from the $\dot{V}O_2$ peak test, isometric handgrip and PEI are reported as mean ± standard deviation (SD).

3.3 Results

3.3.1 Participant demographics

All of the groups were matched for age (*P*=0.73; Table 3.1. page 190), BMI (*P*=0.25; Table 3.1, page 190), $\dot{V}O_2$ peak scores (P=0.97; Table 3.1, page 190) and anaerobic threshold (P=0.75; Table 3.1. page 190). As predicted, daytime ambulatory SBPs were different between groups (*F*=26.57; *P*=<0.0001; Table 3.1, page 190). Most importantly, the treated-controlled hypertension group had a similar daytime systolic ABPM result compared to normotension (125±7 mm Hg vs. 120±9 mmHg; *P*=0.37). Additionally, treated-uncontrolled hypertensive individuals (145±12 mm Hg) had higher daytime ambulatory SBP when compared to normotensive (120±9 mmHg; *P*=<0.0001) and treated-controlled hypertensives (125±7 mmHg; *P*=<0.0001). Similarly, untreated hypertensives (145±10 mmHg) had a higher daytime ambulatory SBP when compared to normotensive (120±9 mmHg; *P*=<0.0001) and treated-controlled hypertensives

(125±7 mmHg; *P*=<0.0001). Treated-uncontrolled (145±12 mmHg) and untreated hypertensive (145±10 mmHg) individuals had a similar daytime ambulatory SBP (*P*=0.99). Daytime DBP (*F*=8.89; *P*=<0.0001), MAP (*F*=17.53; *P*=<0.0001) and PP (*F*=9.13, *P*=<0.0001) showed a similar pattern between groups (see Table 3.1, page 190). There were no group differences in daytime ambulatory HR (*F*=1.2; *P*=0.32). Similar to daytime systolic ABPM there was also a difference in night-time ambulatory SBP monitoring (*F*=15.35; *P*=<0.0001; Table 3.1, page 190). A Tukey post hoc test showed that night-time ambulatory SBP was similar between normotension and treated-controlled hypertension (108±10 vs. 112±10 mmHg respectively; *P*=0.68). Treated-uncontrolled hypertensives (130±12 mm Hg) had a higher night-time ambulatory SBP when compared to normotensive (108±10 mm Hg; *P*=<0.0001) and treated-controlled hypertensive (112±10 mmHg; *P*=0.002) individuals. Untreated hypertensives (129±12 mmHg) had a higher night-time ambulatory SBP when compared to normotensive (108±10 mmHg; *P*=<0.0001) and treated-controlled hypertensives (112±10 mmHg; *P*=0.001). Treated-uncontrolled (130±12 mmHg) and untreated hypertensives (129±12 mmHg) had a similar night-time ambulatory SBP (*P*=0.99). Similar results were found for MAP (*F*=12.39;*P*=<0.0001;Table 3.1, page 190) and PP (*F*=5.56;*P*=0.002;Table 3.1, page 190) but there were no group differences for night-time ambulatory DBP (*F*=1.82 *P*=0.15; Table 3.1, page 190) or HR (*F*=1.13;*P*=0.35; Table 3.1, page 190). Group averages for clinic BP measurements are shown Table 3.1 (page 190). The antihypertensive medications that participants were taking are shown in Table 3.1 (page 190) and Table 3.2 (page 192).

3.3.2 $\dot{V}O_2$ Peak Test

A significant interaction effect was found between the percentage of $\dot{V}O_2$ peak and the absolute change in SBP (F (15, 275) = 4.937; P=<0.0001; Figure 3.5, page 200). The Tukey post hoc test revealed that that absolute increase in SBP was similar between all groups at 0-25 and 26-50% $\dot{V}O_2$ peak testing (P=>0.05). However, at 51-75% $\dot{V}O_2$ peak testing, treated-uncontrolled (45±14 mmHg) and treated-controlled hypertensives (42±13 mmHg) had a similar change in absolute SBP (P=0.9) that was elevated compared to normotensive individuals (29±17 mmHg; P=0.009 and P=0.048). Untreated hypertensive (42±16 mmHg) and normotensive (29±17 mmHg) individuals had a similar absolute increase in SBP at 51-75% $\dot{V}O_2$ peak (P=0.09). Similarly, at 76-100% $\dot{V}O_2$ peak the treated-uncontrolled (74±22 mmHg), treated-controlled (65±12 mmHg) and untreated hypertensives (68±20 mmHg) had a similar absolute change in SBP (P=>0.05) which was elevated compared to the normotensive group (46±17 mmHg; P=<0.0001, P=0.0007, and P=0.0003 respectively). Similar results were found for peak exercise (Figure 3.5, page 200). The results were similar when assessing the absolute SBP and percentage change in SBP (Figure 3.6 and 3.7, pages 202-204). However, the absolute resting SBP prior to the $\dot{V}O_2$ peak test was not different between the groups (P=>0.05; Figure 3.6, page 202). The SBP/min was different between groups (F=5.762, P=0.002). The Tukey post hoc test showed that the SBP/min was similar between treated-uncontrolled, controlled and untreated hypertensives but was elevated when compared to the normotensive group (8±2,8±1, 8±3 vs. 5±2 mmHg respectively; P=0.02, P=0.002 and P=0.02 respectively).

The absolute change in DBP during exercise was different between groups (*F* (15,275) =3.082, *P*=0.001). The Tukey post hoc test found that the absolute change in DBP was similar between the groups during 0-25 and 26-50% $\dot{V}O_2$ peak (P=>0.05; Figure 3.5, page 200). However, at 51-75% $\dot{V}O_2$ peak the treated-controlled hypertensive group (10±19 mmHg) had an increased absolute change in DBP when compared to normotensives (1±7 mmHg; *P*=0.02). In addition, the absolute change in DBP was similar between treated-uncontrolled (7±9 mmHg) and treated-controlled (10±19 mmHg) hypertension (*P*=0.89) but was elevated when compared to untreated hypertension (-2±8 mmHg; *P*=0.027 and *P*= 0.004 respectively). Normotensive (1±7 mmHg) and untreated hypertensive (-2±8 mmHg) individuals had a similar absolute change in DBP at 51-75% $\dot{V}O_2$ peak (*P*=0.83). At 76-100% $\dot{V}O_2$ peak the treated-uncontrolled hypertension (18±14 mmHg) group had a larger increase in absolute change in DBP when compared to normotensives (5±8 mmHg; *P*=0.0005). Treated-uncontrolled (18±14 mm Hg) and treated-controlled (12±12 mmHg) hypertension had a similar change in absolute DBP (*P*=0.29), but both were exaggerated when compared to untreated hypertension (2±10 mm Hg; *P*=<0.0001 and *P*=0.02 respectively). Again, the absolute change in DBP was similar between normotensives (5±8 mmHg) and untreated hypertensives (2±10 mmHg) at 76-100% $\dot{V}O_2$ peak (*P*=0.78). Similar results were attained at peak exercise (Figure 3.5, page 200). In addition, similar results were found for the absolute change in MAP and PP during the $\dot{V}O_2$ peak (F (15,275) = 4.807; *P*=<0.0001 and (F (15,275) = 3.132; *P*=<0.0001 respectively; Figure 3.5, page 200).

The absolute change in HR during the $\dot{V}O_2$ peak test was different between the groups (F (15,275) = 2.032, P=0.01; Figure 3.5, page 200). The Tukey post hoc test revealed that there was no difference between the groups up to 76-100% $\dot{V}O_2$ peak, where untreated hypertensives (72±19 beats/min) had an exaggerated rise in absolute HR compared to treated-uncontrolled hypertension (61±14 beats/min; P=0.047). At peak exercise, both treated-controlled (80±13 beats/min) and untreated hypertension (83±19 beats/min) had a similar rise in absolute HR (P=0.91) that was exaggerated when compared to normotension (70±14 beats/min; P= 0.04 and P= 0.01). In addition, at peak exercise, both treated-controlled (80±13 beats/min) and untreated hypertension (83±19 beats/min) had an exaggerated change in absolute HR when compared to treated-uncontrolled hypertension (67±16 beats/min; P=0.003 and P=0.001). Normotensive (70±14 beats/min) and treated-uncontrolled (67±16 beats/min) hypertensives had a similar change in absolute HR at peak exercise (P=0.86).

Tidal volume and breathing frequency were similar between all groups during baseline and during the $\dot{V}O_2$ peak test (F(15,330)=0.218, P=0.99 and F(15,330) = 0.88, P=0.59 respectively; Figure 3.8, page 206). Similarly, minute ventilation was also similar between the groups during baseline and the $\dot{V}O_2$ peak test (F (15,330) = 0.29, P=0.99; Figure 3.8, page 206). RER was similar between normotensives (1.21±0.16), treated uncontrolled (1.16±0.12), treated controlled (1.21±0.14) and untreated hypertensives (1.22±0.13) (F(15,330)=0.91; P=0.55) at peak exercise (Figure 3.8, page 206).

The VE/V_ECO_2 slope was different between the groups (F=2.97; P=0.04). A Tukey multiple comparison post-hoc test found that untreated hypertensives (33±4) had an elevated VE/V_ECO_2 slope when compared to normotensive individuals (29±4; P=0.04). However, the VE/V_ECO_2 slope was similar between treated controlled hypertension (32±4) and normotensive individuals (29±4; P=0.12). The VE/V_ECO_2 slope was also similar between treated uncontrolled hypertension (30±4) and normotension (29±4; P=0.93). There was no correlation between the VE/V_ECO_2 slope and the change in SBP (mmHg) at moderate (51-75% $\dot{V}O_2$ peak testing) (r=0.19; P=0.41; Figure 3.9, page 209) or at peak exercise (r=-0.06; P=0.66; Figure 3.9, page 209).

3.3.3 Metaboreflex testing

Two of the 16 treated-controlled hypertensives withdrew between visit one and two. 14 treated-controlled hypertensives therefore completed isometric handgrip and PEI.

3.3.3.1 Baseline

The absolute SBP as measured by the Finapres was similar between normotensive (129±19 mmHg), treated uncontrolled (140±13 mmHg), treated controlled (123±25) and untreated hypertensives (130±26 mmHg) (F(15,265)=1.858; P = 0.15). Similar results were found for baseline absolute DBP between normotensives (57±12 mmHg) treated uncontrolled (58±9 mmHg), treated controlled (56±15) and untreated hypertensives (60±17 mmHg) (F(15,265)= 0.2547; P = 0.86). Similar results were found for MAP and PP. In

addition, there were no differences between normotensives (61±9 beats/min), treated uncontrolled (62±13 beats/min), treated controlled (60±10 beats/min) and untreated hypertension (58±9 beats/min) in baseline absolute HR (*F*(15,265)=0.2482*; P*=0.86). Breathing frequency was also comparable among normotensives (13±3 breaths/min), treated uncontrolled (14±2 breaths/min), treated controlled (13±2 breaths/min) and untreated hypertensives (13±3 breaths/min) (*F*(15,265)=0.68; *P*= 0.57).

3.3.3.2 HRV

3.3.3.2.1 Spectral analysis

No differences were found for either the LF/HF ratio, LF (nu), HF (nu), LF (ms^2) or HF (ms^2) between normotensive, treated controlled, treated uncontrolled or untreated hypertension during the baseline period (P=>0.05; Table 3.3, page 193).

3.3.3.2.2 Time-domain analysis

No differences were found for either the SDDR (ms), RMSDD (ms) or the pRR50 (%) between normotensive, treated controlled, treated uncontrolled or untreated hypertension during baseline (P=>0.05; Table 3.3, page 193).

3.3.3.3 Spontaneous cardiac baroreflex sensitivity

There were no differences in the gain of the cardiac baroreflex sensitivity at baseline between the groups (*P*=>0.05; Table 3.4, page 194). However, during

baseline only 64% of untreated hypertensives, 69% of treated uncontrolled, 86% of treated controlled and 94% of normotensives had 3 or more baroreflex mediated ramps (Table 3.4, page 194). Untreated hypertensives had significantly more baroreflex sequence ramps (total) when compared to normotensives (*P*=0.035), treated controlled (*P*=0.02) and treated uncontrolled (*P*=0.049) (Table 3.4, page 194). The total ramps (baroreflex and non-baroreflex ramps) were not different between groups and can be found in Table 3.4 (page 194). In addition, untreated hypertensives had a higher baroreflex effectiveness index when compared to normotension (*P*=0.0004), treated uncontrolled (*P*=0.007) and treated controlled (*P*=0.0004) (Table 3.4, page 194). However, during baseline the baroreflex effectiveness index could only be assessed in 64% of untreated hypertensives, 69% of treated uncontrolled, 86% of treated controlled and all (100%) of the normotensives (Table 3.4, page 194).

3.3.3.4 Isometric handgrip

The absolute change in SBP, DBP, MAP, PP and HR during isometric handgrip exercise and PEI can be seen in Figure 3.10 (page 210). Figure 3.11 (page 212) shows a typical absolute change in SBP during isometric handgrip and during PEI from one individual from each group. There was a significant interaction effect during isometric handgrip for the absolute change in SBP (F (15,265) = 4.222; P = <0.0001). A post-hoc Tukey test revealed that during 0-30s and 30-60s of isometric handgrip exercise testing, treated controlled and treated uncontrolled hypertensives had a similar absolute change in SBP (*P*=0.83 and *P*=0.14 respectively). Treated-controlled hypertensives had an exaggerated

change in absolute SBP compared to normotensives during 0-30s and 30-60s of isometric handgrip (*P*=0.006 and *P*=0.02 respectively). Treated-uncontrolled hypertensives also had an exaggerated change in absolute SBP compared to normotensives (*P*=0.0001 and *P*=<0.0001 respectively). Untreated hypertensives had a similar change in absolute SBP when compared to normotensives during both 0-30 and 30-60s of isometric handgrip (*P*=0.41 and *P*=0.24 respectively). There was no significant interaction effect for the groups for the absolute increase in DBP during isometric handgrip (*F* (15,265) = 1.701; *P* = 0.051). A significant interaction effect was noted for the absolute change in MAP and PP during isometric handgrip (*F* (15,265) = 3.021, *P*=0.0002 and *F* (15,265) = 2.665, *P*=0.0009). Tukey post-hoc tests revealed similar results compared to the absolute change in SBP for MAP and PP (Figure 3.10, page 210). There was also no significant difference noted for the absolute increase in HR during isometric handgrip (*F* (15,265) = 1.478, *P*=0.11).

There was a significant interaction effect for the absolute SBP during isometric handgrip exercise (*F*(15,265) = 4.16; *P*=<0.0001). A Tukey post test revealed that treated uncontrolled hypertensives had a higher absolute SBP compared to normotensives (*P*=0.01) and treated controlled hypertension (*P*=0.048) during 0-30s of isometric handgrip. Similar results were found during 30-60s of isometric handgrip exercise. No other significant differences were found between groups (*P*=>0.05). No significant differences were found for absolute DBP (*P*=>0.05) and MAP (*P*=>0.05) during 0-30 or 30-60s of isometric handgrip exercise. However, a significant interaction effect was found for absolute PP (*F*(15,265) = 2.589; *P*=0.001). Similar to SBP, treated uncontrolled hypertensives had a higher

absolute PP compared to normotensives (*P*=0.03) and treated controlled hypertension (*P*=0.04) during 0-30s and 0-60s of isometric handgrip. No differences were found for absolute HR during isometric handgrip exercise at 0-30s or 30-60s of isometric handgrip exercise (*P*=>0.05). Finally, there were no significant differences for absolute breathing frequency between groups during 0-30 or 30-60s of isometric handgrip exercise (*P*=>0.05), indicating that participants continued a normal breathing pattern during isometric handgrip exercise.

3.3.3.5 Post-exercise ischemia

A significant interaction effect was found the absolute change in SBP during PEI (F (15,265) = 4.222; P = <0.0001). A Tukey post-hoc test revealed that during the first epoch of PEI treated controlled, treated uncontrolled and untreated hypertension had an exaggerated absolute change in SBP compared to normotension (*P*=<0.0001, *P*=0.0142 and *P*=0.0009). Similar results were seen for the second and third epoch of PEI (see Figure 3.10, page 210). There was not a significant interaction effect for the absolute increase in DBP during the three epochs of PEI (F (15,265) = 1.701; P = 0.051). A significant interaction effect was noted for the absolute change in MAP and PP during the three epochs of PEI (F (15,265) = 3.021, *P*=0.0002 and F (15,265) = 2.665, *P*=0.0009). A Tukey post-hoc test revealed comparable results for MAP and PP compared to SBP (see Figure 3.10, page 210). Similar to isometric handgrip no difference was observed for the absolute change in HR during any of the three epochs of PEI (F (15,265) = 1.478, *P*=0.11).

There was a positive correlation between the change in SBP (mmHg) during metaboreflex isolation and the peak change in SBP (mmHg) during $\dot{V}O2$ peak testing (r = 0.36; R^2 = 0.13; *P*=0.007; see Figure 3.12, page 213). There was no correlation between spontaneous cardiac baroreflex sensitivity and the rise in SBP during PEI (r=-0.1887; *P*=0.29).

There was also a significant interaction effect for the absolute SBP during all epochs of PEI (*F*(15,265) = 4.16); *P*=<0.0001). A Tukey post test revealed that during PEI1, PEI2 and PEI3 treated uncontrolled hypertensives had a higher absolute SBP compared to normotensives (*P*=<0.05) and treated controlled hypertension (*P*=<0.05). No other significant differences were found between groups (*P*=>0.05). There were no significant differences for absolute DBP or MAP (*P*=>0.05) during PEI1, PEI2 or PEI3. However, a significant interaction effect was found for absolute PP during PEI (*F*(15,265) = 2.589); *P*=0.001). Treated uncontrolled hypertensives had a higher absolute PP during PEI1, PEI2 and PEI3 compared to normotensives (*P*=<0.05) and treated controlled hypertensives (*P*<0.05*)*. No other differences were found for PP during PEI1, PEI2 or PEI3 (*P*=>0.05). No differences were found for absolute HR during PEI (*P*=>0.05). Similar to isometric handgrip exercise there were no significant differences for absolute breathing frequency between groups during PEI1, PEI2 and PEI3 (*P*=>0.05).

3.3.3.6 Recovery

There were no differences between normotension, treated uncontrolled, treated controlled or untreated hypertension for the change in SBP, DBP, MAP, PP or HR from baseline during R1, R2, R3, R4 or R5 following metaboreflex testing (*P*=>0.05; Figure 3.13, page 214). Similarly, no differences were found for absolute SBP, DBP, MAP or HR during R1, R2, R3, R4 or R5 following metaboreflex testing (*P*=>0.05). However, absolute PP was significantly elevated in treated uncontrolled hypertensives when compared to normotensives, treated controlled and untreated hypertensives during R1, R2, R3, R4 and R5 (*P*=<0.05).

3.3.3.7 HRV during recovery

3.3.3.7.1 Spectral analysis

No differences were found for either the LF/HF ratio, LF (nu), HF (nu), LF (ms^2), HF (ms^2) between normotensive, treated controlled, treated uncontrolled or untreated hypertension during recovery (*P*=>0.05; Table 3.3, page 193). In addition, no differences were found when comparing baseline to recovery for LF/HF ratio, LF (nu), HF (nu), LF (ms^2), HF (ms^2) to recovery (*P*=>0.05; Table 3.3, page 193).

3.3.3.7.2 Time-domain analysis

No differences were found for either the SDDR (ms), RMSDD (ms) or the pRR50 (%) between normotensive, treated controlled, treated uncontrolled or untreated hypertension during recovery (*P*=>0.05; Table 3.3, page 193). In addition, no

differences were found when comparing baseline to recovery for time-domain HRV analysis (SDDR (ms), RMSDD (ms) or the pRR50 (%) (*P*=>0.05; Table 3.3, page 193).

3.3.3.7.3 Spontaneous cardiac baroreflex sensitivity

There were no differences in the gain of the cardiac baroreflex sensitivity during recovery between the groups (*P*=>0.05; Table 3.3, page 193). However, during recovery only 64% of untreated hypertensives, 75% of treated uncontrolled, 71% of treated controlled and 94% of normotensives had 3 or more baroreflex mediated ramps (*P*=>0.05; Table 3.3, page 193). There were no differences in the baroreflex sequences ramps (total) between groups (*P*=>0.05; Table 3.3, page 193). The total ramps (baroreflex and non-baroreflex ramps) were not different between groups (*P*=>0.05) and can be found in Table 3.3 (page 193). In addition, there were no differences in the baroreflex effectiveness index between groups during recovery (*P*=>0.05; Table 3.3, page 193). However, during recovery the baroreflex effectiveness index could only be assessed in 64% of untreated hypertensives, 75% of treated uncontrolled, 71% of treated controlled and 94% of normotensives (Table 3.3, page 193).

3.4 Discussion

The main finding of this study is that individuals with treated controlled hypertension have an excessive rise in absolute SBP during moderate and peak dynamic exercise compared to normotensive individuals, despite having similar resting BP. In addition, individuals with treated controlled hypertension had a

disproportionate change in BP during isolation of the metaboreflex, which was higher than normotensives. This suggests that the metaboreflex plays a part in mediating this abnormal BP response to exercise in treated and untreated hypertension. Most concerning is that this occurs regardless of antihypertensive treatment and adequate control of resting BP.

3.4.1 Blood pressure during dynamic exercise and cardiovascular risk

The current goal of the clinician is to normalise resting BP to guidelines targets and lower the risk of CV events in patients. The results of this study and other studies suggest that this routine clinical practice needs to be reassessed. This is especially concerning as the BP response to exercise has been associated with acute myocardial infarction (Mittleman and Siscovick, 1996, Willich et al., 1993) and subarachnoid haemorrhage (Anderson et al., 2003, Schievink et al., 1989). Additionally, it is an independent risk factor for long term risk of adverse CV events (Kjeldsen et al., 1997, Kjeldsen et al., 2001), any type of stroke (Kurl et al., 2001, Laukkanen et al., 2006), left-ventricular hypertrophy (Ren et al., 1985, Papademetriou et al., 1989), myocardial infarction (Laukkanen et al., 2006, Kjeldsen et al., 1997, Kjeldsen et al., 2001, Mundal et al., 1996, Filipovsky et al., 1992, Kohl et al., 1996) and total mortality (Filipovsky et al., 1992, Kohl et al., 1996, Fagard et al., 1996). This study highlights that an exaggerated rise in BP during dynamic and isometric exercise may be a factor contributing to the increased CV risk in treated controlled hypertension when compared to normotensives (Lawlor et al., 2011, Almgren et al., 2005, Brown et al., 2013). Clinicians may need to consider exercise testing as part of the screening protocol

when diagnosing and treating patients with hypertension. This may help to fully highlight the CV risk of the patient.

The VE/V_EVCO_2 slope was increased in patients with untreated hypertension when compared to normotensives. The peripheral chemoreceptors which are located in the aortic and carotid bodies senses changes in body partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2) and pH and relay afferent information to the nucleus tractus solitarius via the carotid sinus and vagus nerve (Guyenet, 2014). Stimulation of the peripheral chemoreceptors leads to increased respiratory drive and efferent SNA (Sinski et al., 2012, Stickland et al., 2008). Increased ventilation during exercise is tightly related to increases in CO_2 production and peripheral chemosensitivity has been associated with an exaggerated VE/V_EVCO_2 slope during exercise in chronic heart failure (Chua et al., 1997, Tomita et al., 2003). Although this has not directly been assessed in hypertension, the results from this study suggest increased peripheral chemoreceptor activity in untreated hypertension (Ponikowski et al., 2001). The VE/V_EVCO_2 slope has added prognostic value during exercise. For example, Arena et al. (2008) reported that in heart failure, an VE/V_EVCO_2 slope >35 is associated with increased risk of an adverse cardiac event. In contrast, Buys et al. (2013) found that a VE/V_EVCO_2 slope >27 was associated with increased risk for the future development of hypertension following surgery for aortic coarctation. The individuals with untreated hypertension in this study had a VE/V_EVCO_2 slope of 33±4 compared to 29±4 in normotension. Similar to this study, a previous study found a VE/V_EVCO_2 slope of 29±4 in healthy control patients (Shen et al., 2015). There is a paucity of research around the

VE/V_EVCO_2 slopes in essential hypertension and more research is needed to understand whether these values are prognostically important. In addition, both treated hypertension groups had similar VE/V_EVCO_2 slopes compared to normotension. Although this is suggestive that antihypertensive medication can influence the sensitivity of the peripheral chemoreceptors during exercise, more research is needed to confirm this.

3.4.2 The Metaboreflex

Interestingly, there was no difference in baseline absolute BPs between the groups prior to metaboreflex testing. It is unclear why there was no difference in baseline BP, especially as there were differences between the groups using clinic and daytime ABPM. However, The Finometer Pro (Finometer, FMS, Netherlands) when measuring absolute SBP fails to meet the guidelines of the Advancement of Medical Instrumentation (AAMI) of a standard deviation of < 8 mmHg between intra-arterial BPs and the device being tested. Indeed, the Finapres may under or over predict BP by as much as ± 4 for SBP. However, the change in SBP using the Finometer Pro has been shown to be more accurate (Parati et al., 1989). The remainder of this Chapter will be focused on the absolute change in SBP from the Finometer Pro.

There was no difference in LF/HF, LF (nu), HF (nu), LF (ms^2), HF (ms^2), pRR50, SDDR or RMSSD components of HRV among the groups at rest or during recovery from metaboreflex testing. Previous research has suggested that the HF (nu) and the RMSSD HRV components are predominantly linked to the PSNS

(Akselrod et al., 1981, Pomeranz et al., 1985). The utility of the LF (nu) and the LF/HF ratio has been questioned because both components are not eliminated following parasympathetic denervation and β-adrenergic receptor blockade (Randall et al., 1991). In addition, there is reported disparity in the literature between the LF component of HRV and the gold standard measurement of SNA, microneurography (Notarius et al., 1999). Previous research in small groups of patents with hypertension has found mixed results regarding the sequence and time-domain measures of HRV relative to normotension (Dassi et al., 1991, Radaelli et al., 1994). However, studies with larger numbers of participants have found that HRV is impaired in hypertension (Singh et al., 1998, Huikuri et al., 1996). Therefore, a larger cohort may have been needed in this study to show differences in HRV among the groups.

Cardiac baroreflex sensitivity has previously been demonstrated to be impaired in patients with hypertension (Zuern et al., 2013, Bristow et al., 1969, Gribbin et al., 1971, Pikkujamsa et al., 1998). However, there was no differences noted in this study. A limitation of the sequencing method to establish cardiac baroreflex sensitivity is that the original research to develop the technique was based on 24-hour readings. A typical 24-hour period typically produces around 80 sequences per hour (Parati et al., 1988). A 10-minute baseline period was used for this study and 94% (15 out of 16) of normotensives, 86% (12 out of 14) of treated uncontrolled, 69% (11 out of 16) of treated controlled and 64% (7 out of 11) of untreated hypertensives had 3 or more baroreflex linked sequences during baseline to assess resting cardiac baroreflex sensitivity (Table 3.3, page 193). This lack of data limits the ability to compare to the previous literature. Potentially,

longer periods of time (e.g. 24 hours) are needed to truly establish differences in cardiac baroreflex sensitivity between groups (Parati et al., 1988). There remained no significant differences in cardiac baroreflex sensitivity during recovery from metaboreflex testing between groups. A similar number of participants had 3 or more baroreflex related sequences following exercise when compared to baseline (Table 3.3, page 193).

3.4.2.1 Metaboreflex testing

It is well established that the metaboreflex is abnormal in animal models of hypertension (Mizuno et al., 2011a, Mizuno et al., 2011b, Mizuno et al., 2013, Sala-Mercado et al., 2013) as well as in older patients with untreated hypertension (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014). This study has extended these findings to older patients with treated controlled and treated uncontrolled hypertension. This heightened metaboreflex sensitivity contributes to the exaggerated BP response to dynamic and isometric exercise in treated hypertension, regardless of BP control. A recent study found that heightened metaboreflex sensitivity in a canine model of hypertension (via partial unilateral renal artery occlusion), led to coronary vasoconstriction that limited the exercise induced rise in CO and augmented peripheral vasoconstriction (Sala-Mercado et al., 2013). Coronary vasoconstriction during exercise may limit O_2 delivery to the myocardium and limit ventricular performance. This would also contribute to the elevated CV risk associated with exercise in hypertension.

In accordance with previous studies, a similar HR response during metaboreflex isolation among the individuals with hypertension and normotension was seen (Delaney et al., 2010, Sausen et al., 2009). During metaboreflex isolation (PEI) where isometric exercise has stopped, it is well documented that despite continued BP elevation, HR returns to baseline values (Crisafulli et al., 2003). Withdrawal of the mechanoreceptors and central command plus intense baroreflex stimulation leads to an increase in parasympathetic activity and a decrease in HR towards baseline levels, despite heightened SNA maintained by the metaboreflex (Crisafulli et al., 2003, O'Leary, 1993). This phenomenon is known as accentuated antagonism (Uijtdehaage and Thayer, 2000).

The metaboreflex mediated increases in SNA, as measured by microneurography is exaggerated in patients with untreated hypertension (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014). This has also been confirmed during metaboreflex activation in spontaneously hypertensive rats (SHR's) by measuring RSNA (Mizuno et al., 2011b, Mizuno et al., 2011a). Although, microneurography was not done in this study, evidence from previous literature (Delaney et al., 2010, Sausen et al., 2009) would suggest that exaggerated MSNA during metaboreflex activation mediates the BP response to exercise in treated controlled and uncontrolled hypertension.

The findings reported in this study are consistent with some (Greaney et al., 2014, Delaney et al., 2010, Sausen et al., 2009) but not all studies (Rondon et al., 2006) in individuals with untreated hypertension. Interestingly, the mean age of

the participants was above 60 years of age in the studies finding that metaboreflex activity was elevated in untreated hypertension (Greaney et al., 2014, Delaney et al., 2010, Sausen et al., 2009). In contrast, in the study by Rondon et al. (2006), the average age of normotensives and untreated hypertensives was 38±1 and 42±1 years of age respectively. The current consensus is that with increasing age there is a shift towards more oxidative skeletal muscle phenotype leading to a reduction in the metabolic disturbance that occurs with exercise and therefore a reduction in metaboreflex activity (Markel et al., 2003). Interestingly, hypertension is associated with a lower proportion of oxidative muscle fibres in the vastus lateralis when compared to normotension (Hernelahti et al., 2005). In addition, arterial stiffness (Parikh et al., 2016) and MSNA (Hart et al., 2009a, Hart et al., 2009b) increase with age and are both exaggerated further in patients with hypertension (Warnert et al., 2016). These factors may explain why metaboreflex activity is maintained or elevated in older untreated hypertensive individuals.

Activation of the mechanoreflex, using passive hindlimb stretch in SHR's also leads to exaggerated BP and RSNA when compared to Wistar-Kyoto rats (Leal et al., 2008, Mizuno et al., 2011b, Leal et al., 2013). Moreover, BP and MSNA are higher during passive cycling (Velasco et al., 2015) and the first 10 seconds of handgrip exercise (Greaney et al., 2015a) in patients with untreated hypertension. However, the metaboreflex component of the exercise pressor reflex was the sole focus of this Chapter due to the wealth of previous literature in untreated hypertension (Delaney et al., 2010, Sausen et al., 2009, Bruce et al., 1945, Rondon et al., 2006, Greaney et al., 2014). In addition, during exercise of

increasing intensity where the metabolism changes primarily from oxidative metabolism to glycolysis the changes in the concentration of metabolites (e.g. lactate) are likely to augment metaboreflex activity as compared to mechanoreflex activity. Indeed, Kaufman et al. (1983) found that the metaboreflex increases its firing rates with prolonged exercise. In contrast, the mechanoreceptors fire at the onset of exercise but then fire at a reduced rate with continued exercise (Kaufman et al., 1983). The previous literature has shown that PEI isolates the metaboreflex component of the exercise pressor reflex (Kaufman et al., 1984, Alam and Smirk, 1937), whereas it is more difficult to truly isolate the mechanoreceptors, independent of the metaboreflex, central command or the arterial baroreflex.

3.4.3 Mechanisms

The exact mechanisms that mediate altered metaboreflex activity in hypertension are unclear. Increased SNA during exercise is directed to all vascular beds during exercise, including the active skeletal muscle. However, in normotensive individuals the increased SNA is offset in the active skeletal muscle by vasoactive metabolites (Thomas et al., 1997a, Thomas et al., 1994, Thomas et al., 1997b, Thomas and Segal, 2004, Thomas et al., 2003, Thomas and Victor, 1998). This protective mechanism ensures O_2 demand is met with adequate supply during exercise and is known as functional sympatholysis (Thomas et al., 1997a, Thomas et al., 1994, Thomas et al., 1997b, Thomas and Segal, 2004, Thomas et al., 2003, Thomas and Victor, 1998). Animals with experimentally induced hypertension and humans with untreated hypertension exhibit impaired functional

sympatholysis during exercise and this has been shown to be mediated, in part, by a reduced nitric oxide (NO) bioavailability, which is caused by a damaged endothelium and/or increased oxidative stress within the skeletal muscle itself (Zhao et al., 2006, Price et al., 2013, Vongpatanasin et al., 2011). Impaired functional sympatholysis leads to a blunted increase in blood flow to the active skeletal muscle, which may cause larger accumulations in the metabolites that activate the metaboreflex in hypertension (Price et al., 2013, Vongpatanasin et al., 2011, Zhao et al., 2006). It could be speculated that the current first line treatment for hypertension (calcium channel blockers, thiazide-like diuretics and angiotensin-converting enzyme inhibitors (Ahluwalia and Bangalore, 2017) do not: A) selectively inhibit the receptors known to activate the metaboreflex or B) normalise functional sympatholysis/improve blood flow during exercise. This requires future investigation to assess specific effects of individual anti-hypertensive therapies. A recent series of studies found that nebivolol, a β_1-adrenoceptor antagonist with NO potentiating effects improves functional sympatholysis during dynamic handgrip exercise (Price et al., 2013) whereas irbesartan, a angiotensin II receptor antagonist failed to cause any improvements (Vongpatanasin et al., 2011). Novel anti-hypertensive medications need to reduce BP at rest and also dynamic BP control during dynamic and isometric exercise, as well as during other stressors (e.g. mental stress and pregnancy). Although, recent evidence has highlighted key receptors that activate the metaboreflex (Pollak et al., 2014, Light et al., 2008), it is likely that the metaboreflex is more complex and much redundancy has been shown by studies blocking the receptors (Stone et al., 2015). Improving functional sympatholysis and increasing the washout of metabolites that activate the metaboreflex may

provide the best option for the pharmacological treatment of exaggerated BP responses to exercise.

Despite the clear importance of the metaboreflex in untreated hypertension (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014) very little research has assessed the sensitivity/density of the receptors within the skeletal muscle that are known to activate the metaboreflex in hypertension. The only study that has directly looked at this so far was by Mizuno et al. (2011a), they found that expression of TRP_V1 was upregulated in the dorsal horn of the spinal cord in SHR's but not in the skeletal muscle where the afferent nerve endings lie. It was speculated that enhanced phosphorylation of the TRP_V1 receptors on the afferent nerve endings could mediate enhanced metaboreflex activity during exercise in SHR's (Mizuno et al., 2011a).

The $\dot{V}O_2$ peak scores of the participants were within the normal range for their age group (Kaminsky et al., 2015) and all the groups were matched for $\dot{V}O_2$ peak scores. Increasing CV fitness after 8 weeks of aerobic training has been shown to improve functional sympatholysis and BP during one-legged knee extensor exercise (Mortensen et al., 2014). Similarly, in SHR's 12 months of wheel running has been shown to decrease the pressor response and RSNA during activation of the metaboreflex similar to levels seen in Wistar-Kyoto rats (Mizuno et al., 2014a). Therefore, increasing CV fitness could also improve the autonomic response to exercise in hypertension. However, poor adherence to exercise

programs is well reported and therefore methods to improve adherence need to be emphasised (Brand et al., 2014).

Far more attention has been given to SBP during exercise in the literature and it remains unclear as to why the DBP was higher during both moderate (51-75% $\dot{V}O_2$ peak) and peak exercise in both the treated controlled and treated uncontrolled hypertensive group when compared to patients with untreated hypertension and normotension. Normally, DBP remains the same or decreases slightly during exercise, indicative of a decline in vascular resistance (Schultz and Sharman, 2014). In contrast, an exaggerated increase in DBP during exercise highlights an impaired vasodilation of resistance vessels within the skeletal muscle vasculature (Brett et al., 2000) and increased risk of CV events (Chatterjee et al., 1995, Brett et al., 2000). In addition, increased aortic stiffness has been shown to increase SBP and decrease DBP, resulting in elevated PP (Laurent et al., 2001, Laurent et al., 2003). Aortic stiffness has previously been shown to be elevated in untreated hypertension and could be related to the reduction in DBP during exercise (Laurent et al., 2001, Laurent et al., 2003). Furthermore, different antihypertensive medications have different effects on aortic stiffness with ACEi's and ARB's being most effective (Blacher et al., 2005, Boutouyrie et al., 2011, Protogerou et al., 2009). Further research is needed to fully understand these results.

3.4.4 Study limitations

Firstly, adherence to anti-hypertensive medication is typically poor (Herttua et al., 2013, Ong et al., 2007) and it was not assessed whether patients with treated controlled or treated uncontrolled hypertension were taking their medication(s). However, the individuals with treated controlled hypertension had BP under control during daytime ABPM and this would suggest that this group were adherent to their anti-hypertensive medication(s). In addition, participants were asked to record a 24-hour diary during ABPM monitoring and to take note of the time that they took their anti-hypertensive medication(s). Second, the treated controlled and treated uncontrolled hypertension group both started the study taking anti-hypertensive medication, future research will need to assess whether different types of anti-hypertensive medications have a different effect on the BP response to dynamic and isometric exercise. For example, a recent study found that perindopril, a centrally acting angiotensin converting enzyme inhibitor was more effective at lowering SBP and MSNA than a peripherally acting angiotensin converting enzyme inhibitor (captopril) during dynamic exercise (Moralez et al., 2018). Future research will need to confirm whether centrally acting anti-hypertensives are more effective in normalising exercise BP and metaboreflex activity in treated controlled and treated uncontrolled hypertension. Thirdly, PEI may activate a subtype of the group IV afferents that respond to high levels of metabolites that mediate pain (Light et al., 2008, Pollak et al., 2014, Kaufman et al., 1984, Amann and Light, 2015). An increased perception of pain during PEI may have caused an elevated BP response (Sacco et al., 2013). Although the differences in pain perception between the groups during PEI were not assessed in this study, individuals with hypertension have a reduced sensitivity to painful

stimuli (Ring et al., 2008) and all participants received the same occlusion pressure of 240 mmHg.

The metaboreflex was assessed following isometric handgrip exercise with a small muscle mass (forearm) and compared to the BP response to dynamic exercise with a large muscle mass (legs, $\dot{V}O_2$ peak test). The CV response to metaboreflex isolation is different following forearm and leg exercise (Fisher et al., 2013) and although the exact mechanism is not understood, it could be related to differences in skeletal muscle fibre type, sympathetic vasoconstrictor tone and the regulation of functional sympatholysis (Fisher et al., 2013).

3.4.5 Clinical perspectives

A change in SBP of more than 64 mmHg at peak exercise in the general population has been shown to place individuals at increased CV risk (Laukkanen et al., 2006). In our treated controlled group, there was a similar change in SBP of 72 mmHg. Mizuno et al. (2016b) found that patients with treated hypertension who still presented with an exaggerated BP response to exercise had an impaired regression of left ventricular hypertrophy compared to patients with treated hypertension who had a normalised BP response to exercise. Based on this data, future longitudinal studies will need to assess whether exaggerated BP response to exercise in patients with treated-controlled hypertension have an increased CV risk. In terms of the management of hypertension, these results suggest that BP testing should be included in routine clinical practice for treated controlled, uncontrolled and untreated hypertension and this will highlight

individuals at increased CV risk. This study has highlighted that despite BP control at rest, treated controlled hypertensives remain hypertensive during CV stress. This suggests an underlying pathology that is currently not being treated in hypertension with the currently given anti-hypertensive medications. The SPRINT (Systolic Blood Pressure Intervention Trial) found that intensive anti-hypertensive treatment to lower SBP below 120 mmHg conferred greater CV benefits (Wright et al., 2015). The treated controlled hypertensive group in this study had a daytime ambulatory SBP of 125±7, it remains to be seen whether further lowering of SBP along with the SPRINT guidelines would normalise the BP response to exercise and metaboreflex isolation.

3.5 Conclusions

This is the first study to show that adequate control of BP, classified by 24-hour ABPM, fails to normalise the BP response to moderate and peak exercise testing when compared to normotensives. An exaggerated change in SBP during exercise may explain why treated controlled hypertensives are at increased CV risk, despite a similar resting BP, compared to normotensives. In addition, it has been shown that the metaboreflex partially mediates this abnormal response to exercise in treated controlled, uncontrolled and untreated hypertension.

3.6 Tables

Table 3-1 Participant Characteristics.

Participant demographics	Normotension	Untreated Hypertension	Treated-uncontrolled hypertension	Treated-controlled hypertension
N	16	11	16	16
M/F	8/8	6/5	9/7	7/9
Age (Years)	65±5	65±7	66±6	67±6
Height (cm)	172±11	173±12	170±9	167±9
Weight (kg)	70±14	72±14	74±12	72±12
BMI (kg/m^2)	23.4±3.1	24.1±1	25.5±0.91	25.3±0.80
$\dot{V}O_2$ peak (ml/min/kg)	22.6±5.4	23.5±6.8	22.8±7.6	22.3±4.9
AT (%)	74±12	70±11	70±13	70±9
Daytime ABPM				
SBP (mmHg)	120±9	145±10*†	145±12*†	125±7
DBP (mmHg)	73±6	86±13*	86±8*†	77±7
MAP (mmHg)	89±7	106±11*†	105±8*†	93±6
PP (mmHg)	46±6	59±9*†	59±10*†	48±8
HR (beats/min)	73±10	69±5	69±8	68±8
Night-time ABPM				
SBP (mmHg)	108±10	129±12*†	130±12*†	112±10
DBP (mmHg)	64±6	74±10	74±5	65±5
MAP (mmHg)	80±7	93±10*†	93±9*†	82±6
PP (mmHg)	43±7	55±9*	56±11*†	47±7
HR (beats/min)	61±7	59±7	64±8	61±7
Clinic BP measurements				
SBP (mmHg)	126±8	148±17*	158±17*†	138±16
DBP (mmHg)	76±7	86±11*	89±9*†	80±9
MAP (mmHg)	93±6	106±11*	111±11*†	99±10

PP (mmHg)	50±9	62±14	68±15*	58±13
HR (beats/min)	69±11	67±11	64±12	65±11
Anti-Hypertensive Medications				
Median number of anti-hypertensive medications	N/A	N/A	1 (IQR=1-1.75)	2 (IQR=1-2)
Percentage of participants taking anti-hypertensives (by class)				
ACEi (%)	0	0	44	88
ARB (%)	0	0	25	31
CCB (%)	0	0	44	56
α-blocker (%)	0	0	6	6
β-blocker (%)	0	0	6	6
Diuretics (%)	0	0	19	6

N; number, M; male, F; female, BMI; body mass index, $\dot{V}O_2$ peak; peak volume of oxygen inspired, AT; anaerobic threshold (%), ABPM; ambulatory blood pressure monitoring, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, PP; pulse pressure, HR; heart rate, ACEi; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, CCB; calcium channel blocker, IQR; inter-quartile range. * *P*=<0.05 vs. normotension; †*P*=<0.05 vs. controlled hypertension (all one-way ANOVA with a Tukey post-hoc test).

Table 3-2 Anti-hypertensive drug classification.

Treated-controlled hypertension		
Anti-hypertensive class	**Specific drug taken**	**% taking drug**
ARB	Candesartan	8
	Losartan	12
ACEi	Perindopril	4
	Ramipril	20
	Lisinopril	4
CCB	Amlodipine	40
α-blocker	Doxazosin	4
β-blocker	Bisoprolol	4
Diuretics	Bendroflumethiazide	4
Treated-uncontrolled hypertension		
ARB	Candesartan	10
	Losartan	5
ACEi	Ramipril	28
	Enalapril	5
CCB	Amlodipine	18
	Felodipine	10
α-blocker	Doxazosin	5
β-blocker	Bisoprolol	5
Diuretics	Bendroflumethiazide	14

ACEi; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, CCB; calcium channel blocker.

Table 3-3 Heart rate variability (HRV).

Heart rate variability (HRV)								
Spectral analysis								
	Baseline				**Recovery**			
	Normotension	**Treated controlled**	**Treated uncontrolled**	**Untreated**	**Normotension**	**Treated controlled**	**Treated uncontrolled**	**Untreated**
LF/HF ratio	1.30±2.5	1.89±2.89	1.67±1.55	3.37±5.91	1.22±1.23	1.63±1.79	1.26±1.52	2.73±4.79
LF (nu)	38±20	45±25	50±24	50±30	44±22	47±21	40±24	51±22
HF (nu)	56±19	49±22	47±22	44±28	52±20	46±18	51±21	45±21
LF (ms^2)	505±739	437±458	520±635	654±529	627±777	474±541	548±355	1100±622
HF (ms^2)	848±1678	695±961	744±1062	646±631	649±582	452±698	673±685	452±698
Time domain								
SDRR (ms)	42±19	47±33	43±21	59±32	53±22	43±19	60±37	67±29
RMSSD (ms)	33±19	44±41	40±26	56±50	35±17	33±21	56±52	58±46
pRR50 (%)	17±15	24±23	35±31	18±22	14±16	10±16	14±17	28±20

LF/HF ratio; low frequency/high frequency ratio, LF; low frequency, HF; high frequency, SDRR; standard deviation of the RR interval, RMSSD; root mean square of successive RR interval differences, pRR50; percentage of successive RR intervals that differ by more than 50 ms.

Table 3-4 Spontaneous cardiac baroreflex sensitivity.

Spontaneous cardiac baroreflex sensitivity								
Sequence technique								
	Baseline				**Recovery**			
	Normotension	**Treated controlled**	**Treated uncontrolled**	**Untreated**	**Normotension**	**Treated controlled**	**Treated uncontrolled**	**Untreated**
Total with sequences (n)	15/16 (94%)	12/14(86%)	11/16 (69%)	7/11(64%)	15/16 (94%)	10/14(71%)	12/16(75%)	7/11(64%)
Up ramps (n)	33±16	34±18	36±11	38±14	40±12	39±17	41±12	40±22
Down ramps (n)	35±16	34±12	34±12	37±16	42±11	40±13	44±16	40±22
All ramps (n)	69±31	67±28	70±21	75±29	83±22	79±29	85±32	80±43
Sequence ramps up (n)	5±5	2±4	4±5	12±8 ‖ ** $	8±7	8±6	5±6	9±5
Sequence ramps down (n)	4±3	4±4	5±6	10±8	7±6	5±4	8±7	8±4
Sequence ramps all (n)	9±8	6±8	9±11	22±16 ‖ ** $	15±12	10±9	16±13	17±9

Up ramps (gain)	12±9	11±8	11±7	10±3	13±10	11±10	11±7	10±2
Down ramps (gain)	11±6	12±6	11±7	10±3	13±7	9±4	10±7	10±4
All ramps (gain)	12±7	12±7	11±7	10±3	13±8	10±6	10±7	11±3
Baroreflex effectiveness index (BEI)								
Total number of participants for BEI (n)	16/16 (100%)	12/14(86%)	11/16 (69%)	7/11(64%)	15/16 (94%)	10/14(71%)	12/16(75%)	7/11(64%)
BEI (up)	0.17±0.12	0.12±0.07	0.18±0.11	0.35±0.17 || ** $	0.2±0.11	0.130.27±0.10	0.27±0.09	0.21±0.06
BEI (down)	0.16±0.09	0.19±0.12	0.22±0.15	0.42±0.23 || ** $	0.23±0.04 #	0.16±0.09	0.24±0.14	0.27±0.13
BEI (all)	0.15±0.1	0.13±0.09	0.2±0.11	0.38±0.19 || ** $	0.2±0.11	0.14±0.08	0.24±0.11	0.23±0.07

\|\| *P*=<0.05 untreated hypertension vs. normotension, ** *P*=<0.05 treated-uncontrolled hypertension vs. untreated hypertension, $ treated-controlled hypertension vs. untreated hypertension and # *P*=<0.05 treated-controlled hypertension vs. treated-uncontrolled hypertension.

3.7 Figures

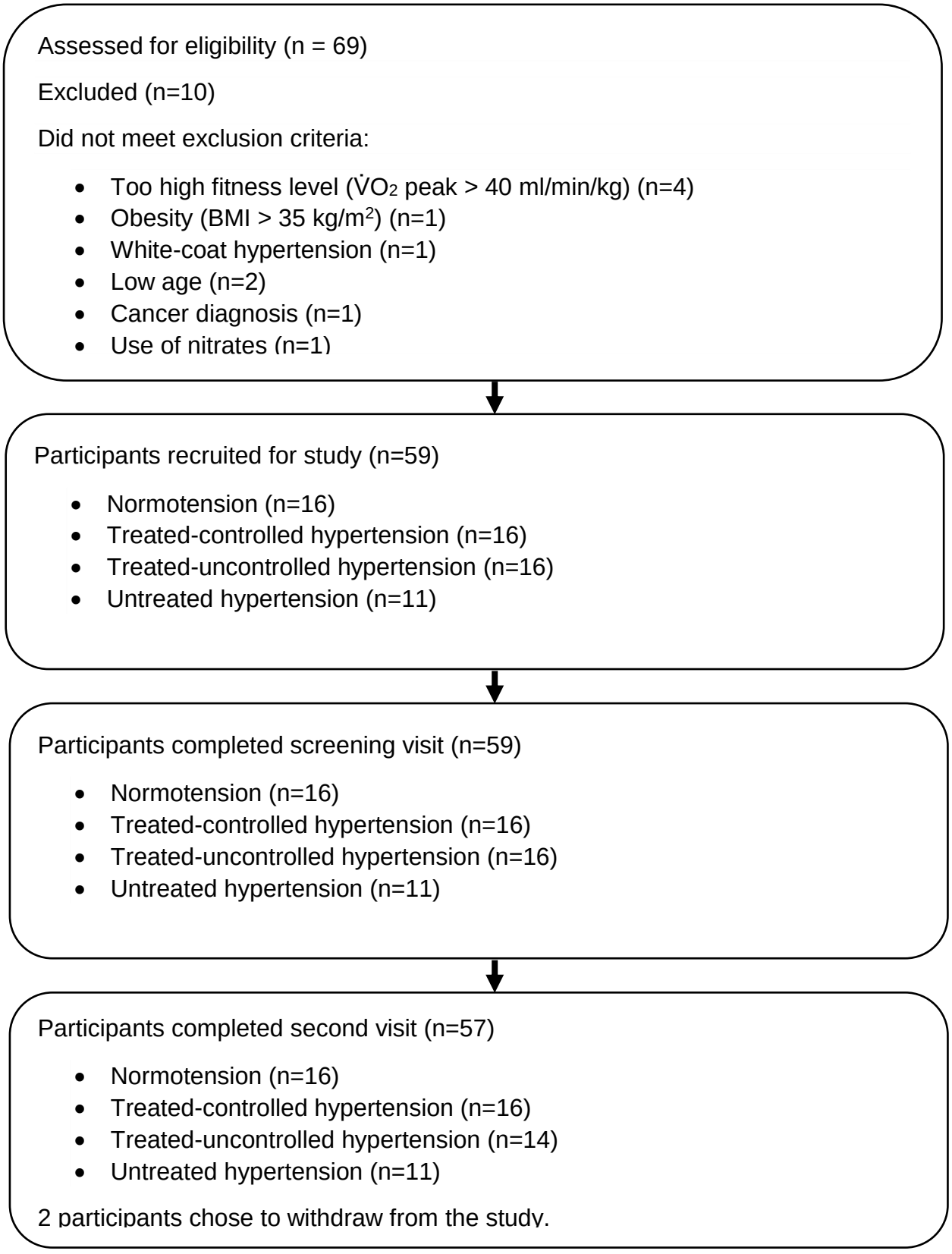

Figure 3-1 Participant recruitment information.

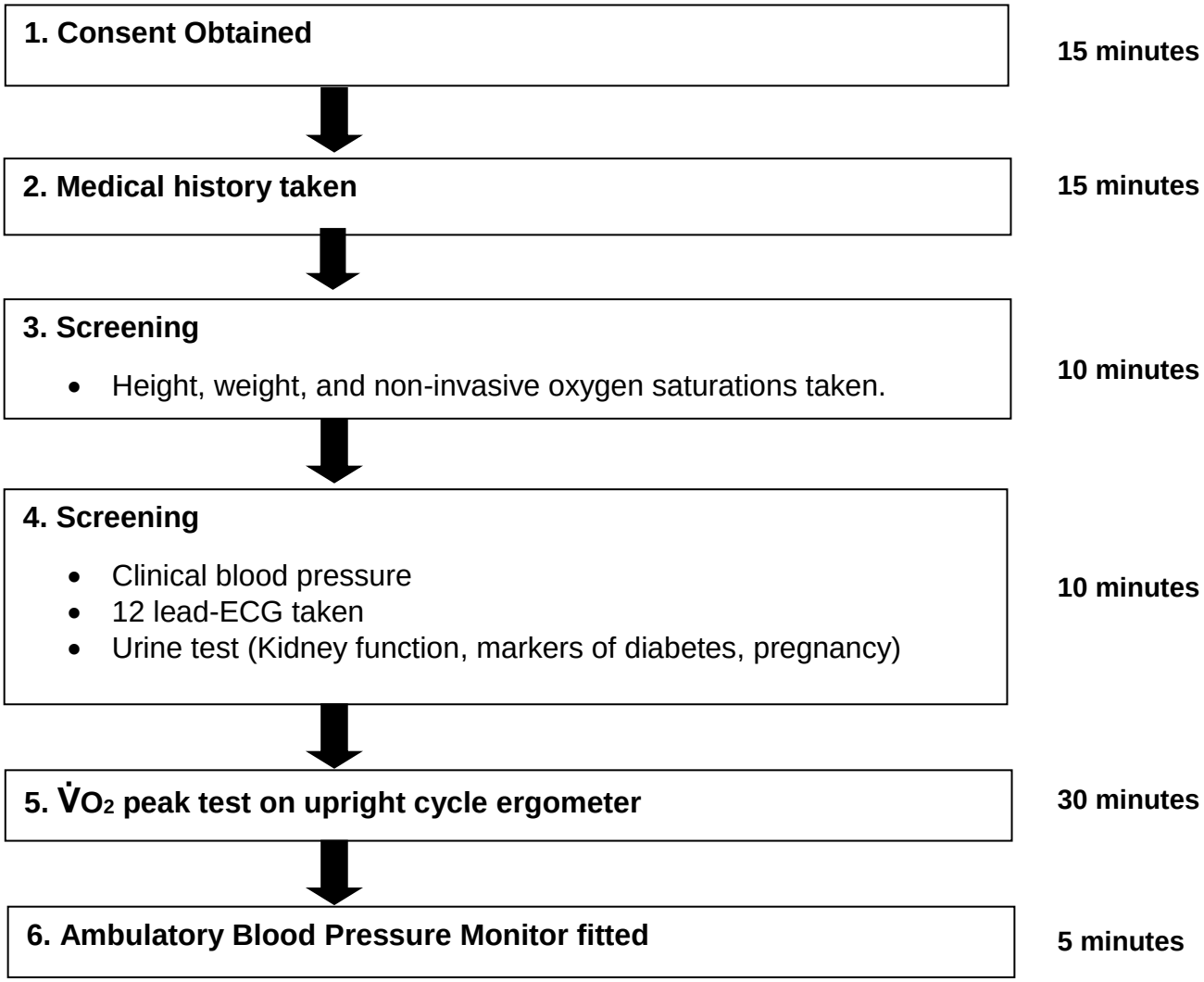

Figure 3-2 Flow chart for visit one to the CRiC.

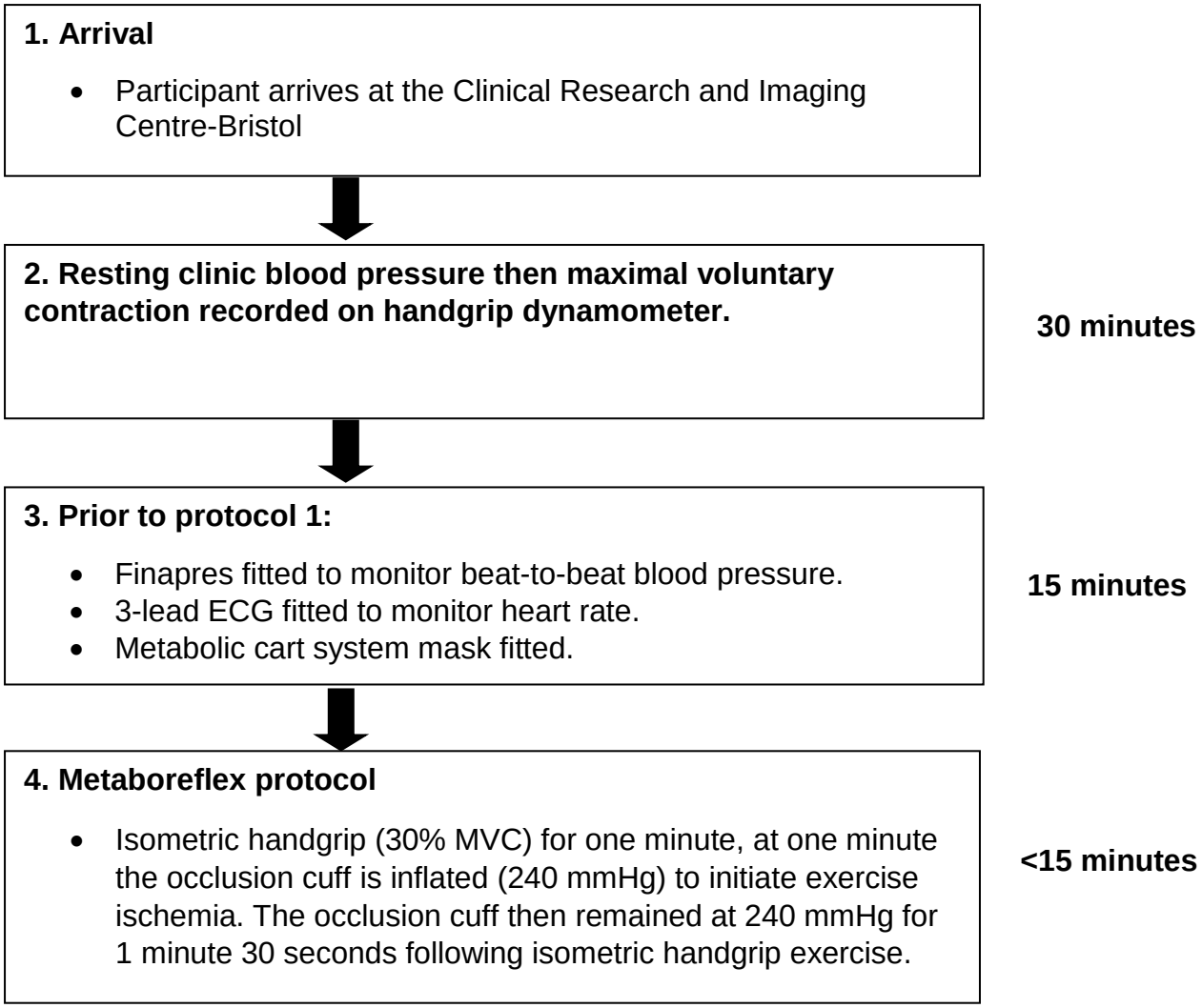

Figure 3-3 Flow chart for visit two to the CRiC.

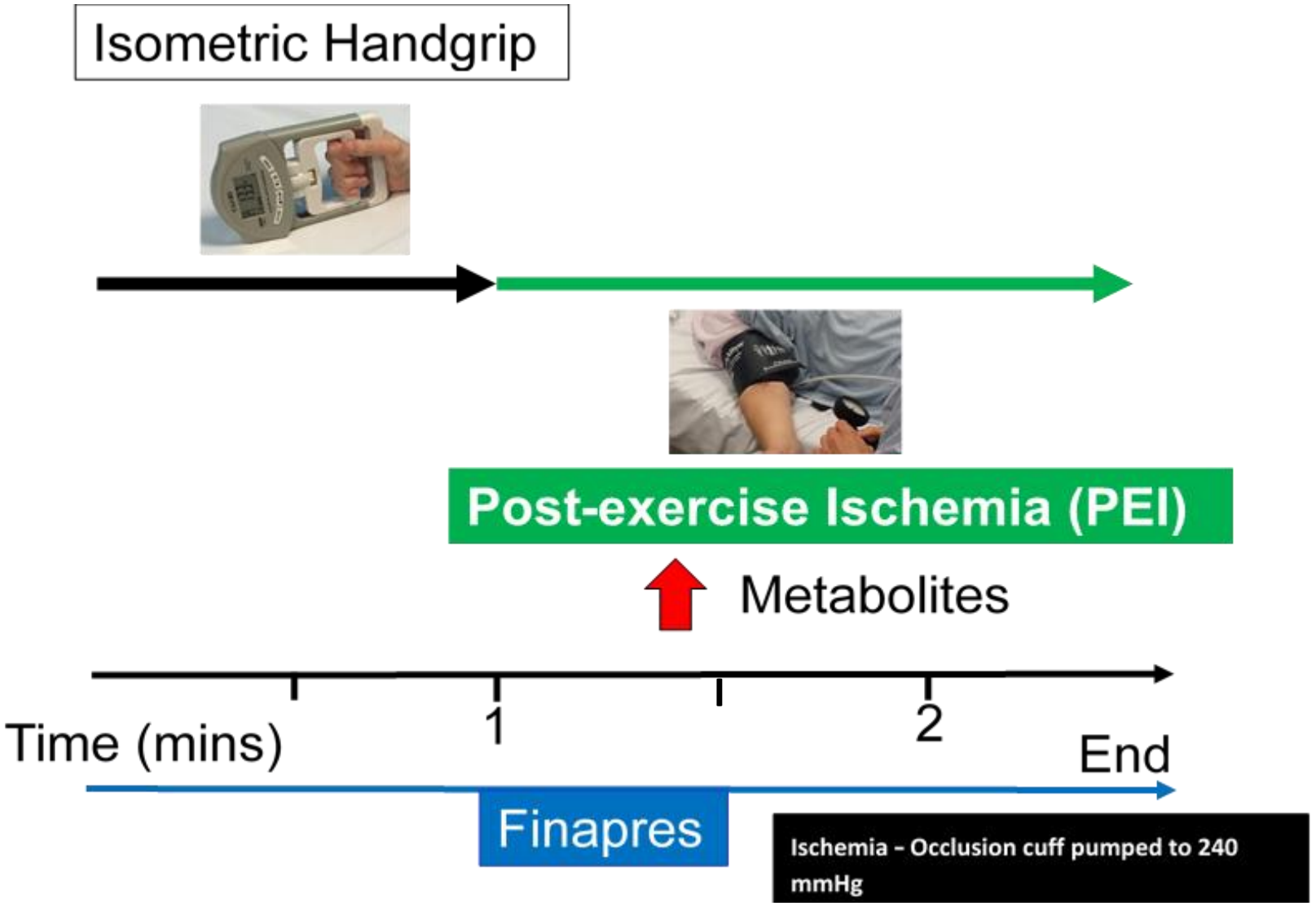

Figure 3-4 A schematic outlining the protocol used to isolate the metaboreflex.

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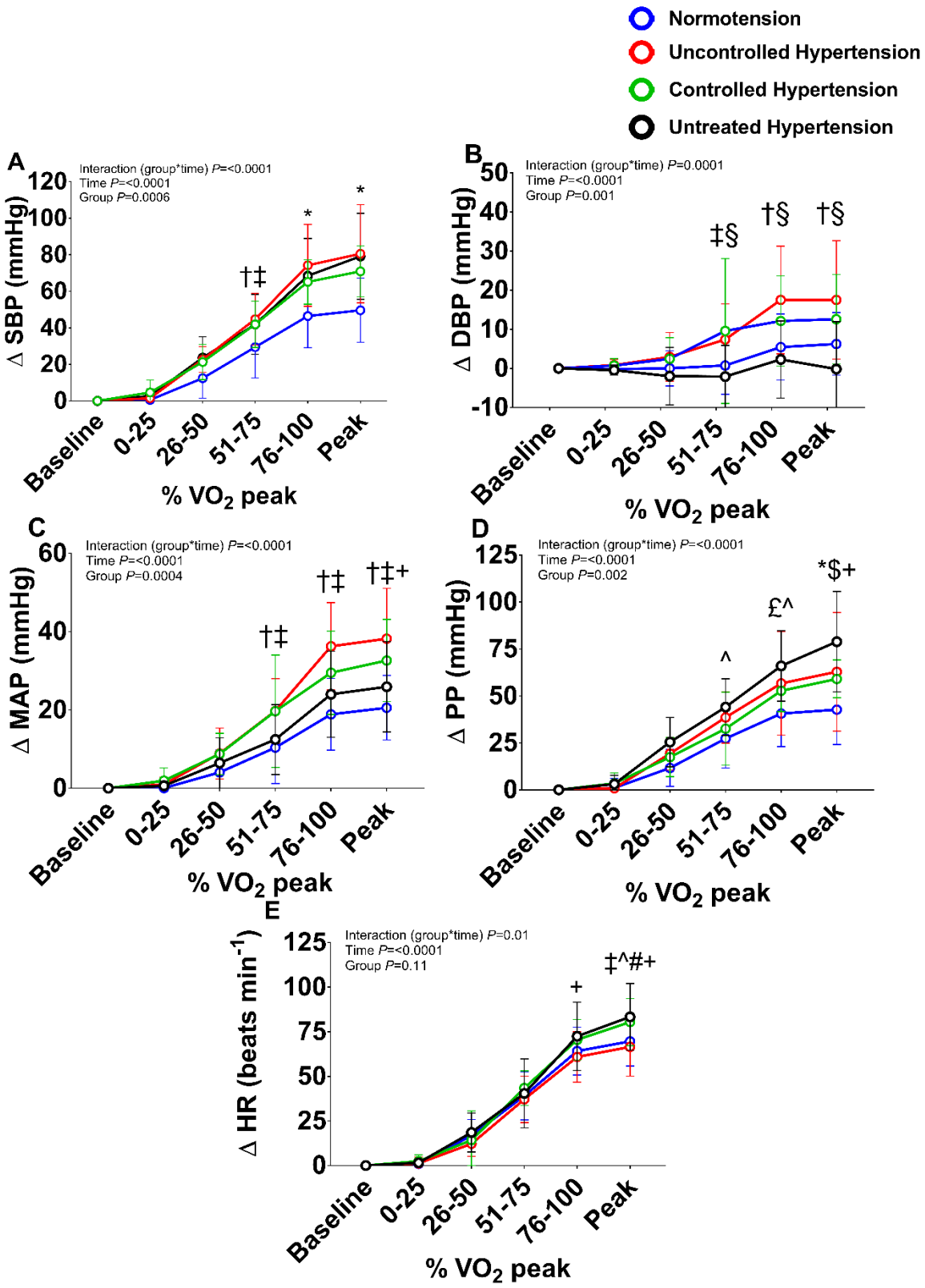


Figure 3-5 The change in haemodynamics from baseline during $\dot{V}O2$ peak testing.

Absolute change from baseline A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) Heart rate (HR). Changes in haemodynamics were calculated at different percentages of $\dot{V}O_2$ peak so that comparisons could be made between the participants. * *P*=<0.05 all groups vs. normotension. † *P*=<0.05 treated-uncontrolled hypertension vs. normotension. ‡ *P*=<0.05 treated-controlled hypertension vs. normotension. § *P*=<0.05 treated-controlled and treated-uncontrolled hypertension vs. untreated hypertension. ^ *P*=<0.05 untreated hypertension vs. normotension. # *P*=<0.05 treated-controlled hypertension vs. treated-uncontrolled hypertension. + *P*=<0.05 treated-uncontrolled hypertension vs. untreated hypertension. $ treated-controlled hypertension vs. untreated hypertension.

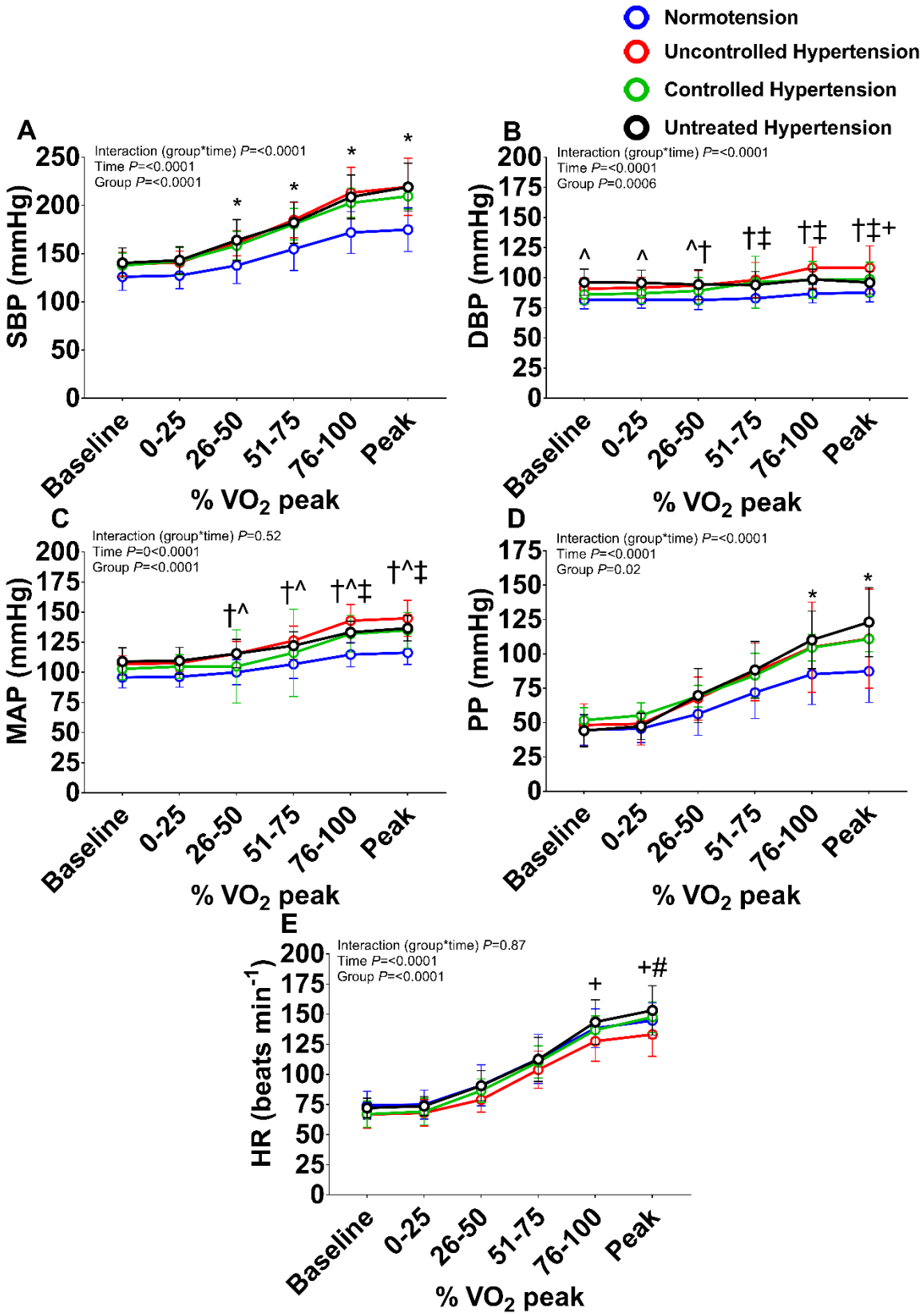


Figure 3-6 Absolute haemodynamics during $\dot{V}O2$ peak testing.

A) systolic blood pressure (SBP), B) Diastolic blood pressure (DBP), C) Mean arterial pressure (MAP), D) Pulse pressure (PP) and E) Heart rate (HR). The cardiovascular response to exercise was split up into differences percentages of $\dot{V}O_2$ peak. * *P*=<0.05 for all groups for normotension. † *P*=<0.05 treated-uncontrolled hypertension vs. normotension. ‡ *P*=<0.05 treated-controlled hypertension vs. normotension. § *P*=<0.05 treated-controlled and treated-uncontrolled hypertension vs. untreated hypertension. ^ *P*=<0.05 untreated hypertension vs. normotension. # *P*=<0.05 treated-controlled hypertension vs. treated-uncontrolled hypertension. + *P*=<0.05 treated-uncontrolled hypertension vs. untreated hypertension. Normotension (blue line), untreated hypertension (black line), treated-uncontrolled hypertension (red line) and treated-controlled hypertension (green line).

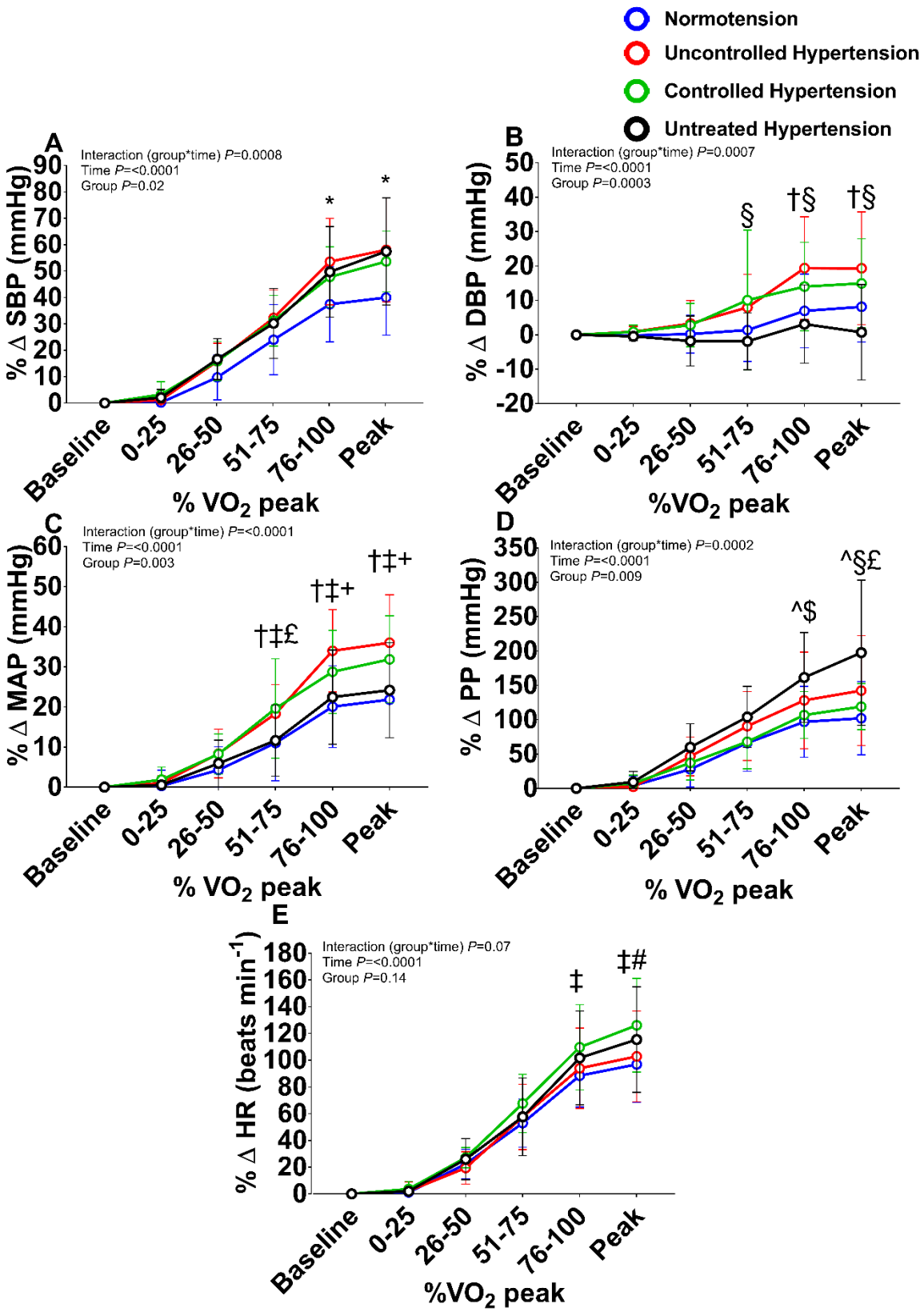


Figure 3-7 The % change in haemodynamics during $\dot{V}O2$ peak testing.

A) systolic blood pressure (SBP), B) Diastolic blood pressure (DBP), C) Mean arterial pressure (MAP), D) Pulse pressure (PP) and E) Heart rate (HR). The data are presented as different percentages of $\dot{V}O_2$ peak. * *P*=<0.05 for all groups for normotension. † *P*=<0.05 treated-uncontrolled hypertension vs. normotension. ‡ *P*=<0.05 treated-controlled hypertension vs. normotension. § *P*=<0.05 treated-controlled and treated-uncontrolled hypertension vs. untreated hypertension. # *P*=<0.05 treated-controlled hypertension vs. treated-uncontrolled hypertension. + *P*=<0.05 treated-uncontrolled hypertension vs. untreated hypertension. £ P=<0.05 treated-controlled hypertension vs. untreated hypertension. ^ *P*=<0.05 untreated hypertension vs. normotension. Normotension (blue line), untreated hypertension (black line), treated-uncontrolled hypertension (red line) and treated-controlled hypertension (green line).

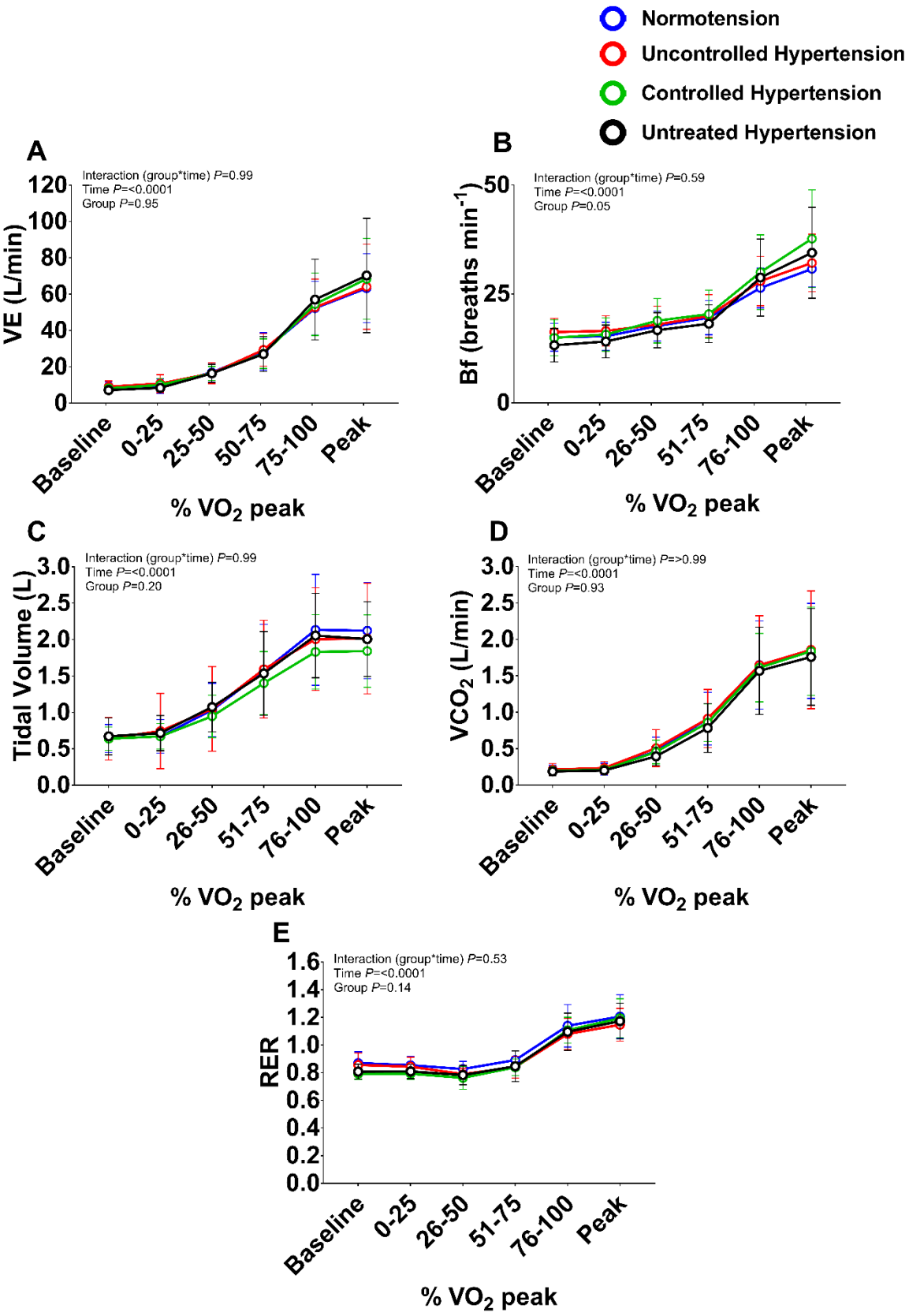
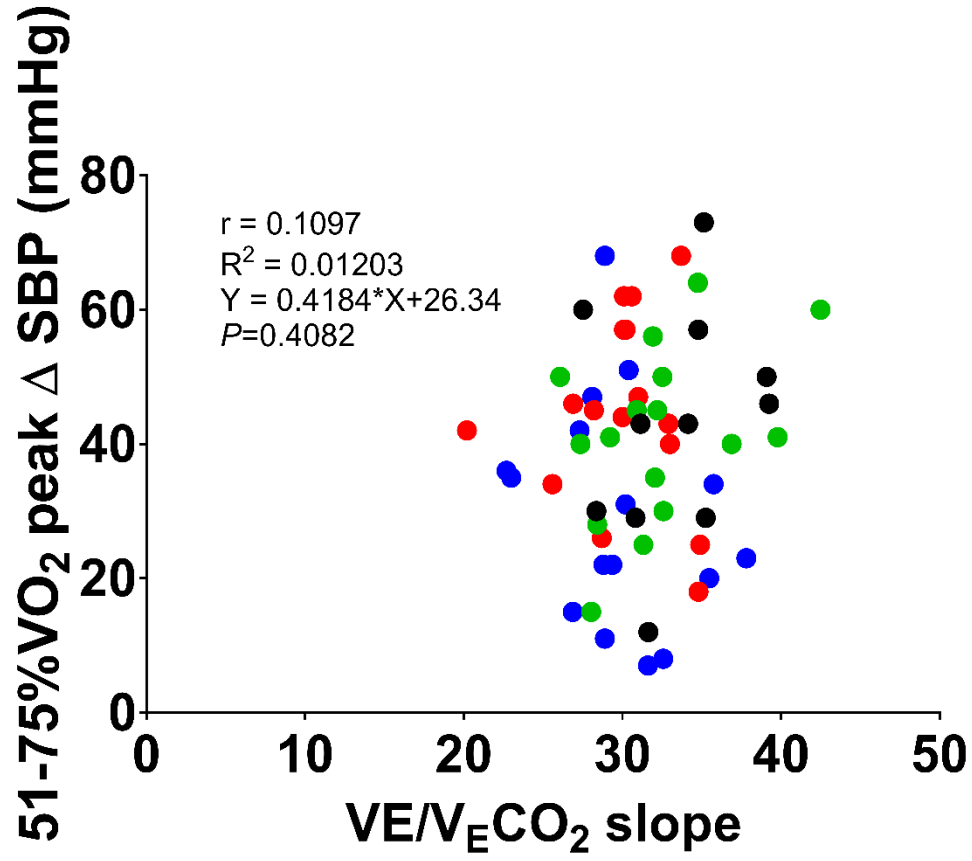


Figure 3-8 The absolute respiratory responses to $\dot{V}O2$ peak test

A) minute ventilation (VE), B) breathing frequency and C) tidal volume D) volume of expired carbon dioxide (VCO2) and E) the respiratory exchange ratio (RER) during $\dot{V}O_2$ peak testing.


51-75%VO2 peak Δ SBP (mmHg)
80
60
40
20
0
r = 0.1097
R2 = 0.01203
Y = 0.4184*X+26.34
P=0.4082
0
10
20
30
40
50
VE/VECO2 slope

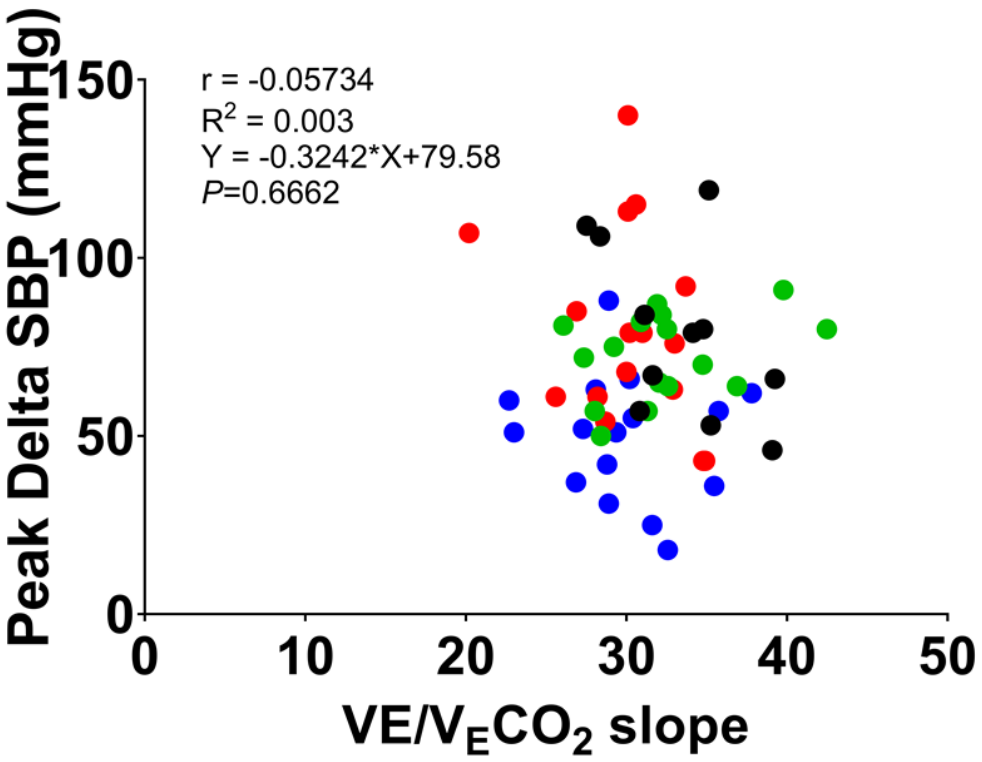

Peak Delta SBP (mmHg)
150
100
50
0
r = -0.05734
R2 = 0.003
Y = -0.3242*X+79.58
P=0.6662
0
10
20
30
40
50
VE/VECO2 slope

Figure 3-9 Relationship between VE/V_EVCO_2 slope and systolic blood pressure during submaximal (51-75 % $\dot{V}O2$ peak testing) and at peak exercise.

The data show that there is no correlation between the VE/V_EVCO_2 slope and the ∆SBP during moderate intensity (51-75% $\dot{V}O_2$ peak testing) or peak exercise. Blue dots, normotension; green dots, treated-controlled hypertension; red dots, treated-uncontrolled hypertension and black dots, untreated hypertension.

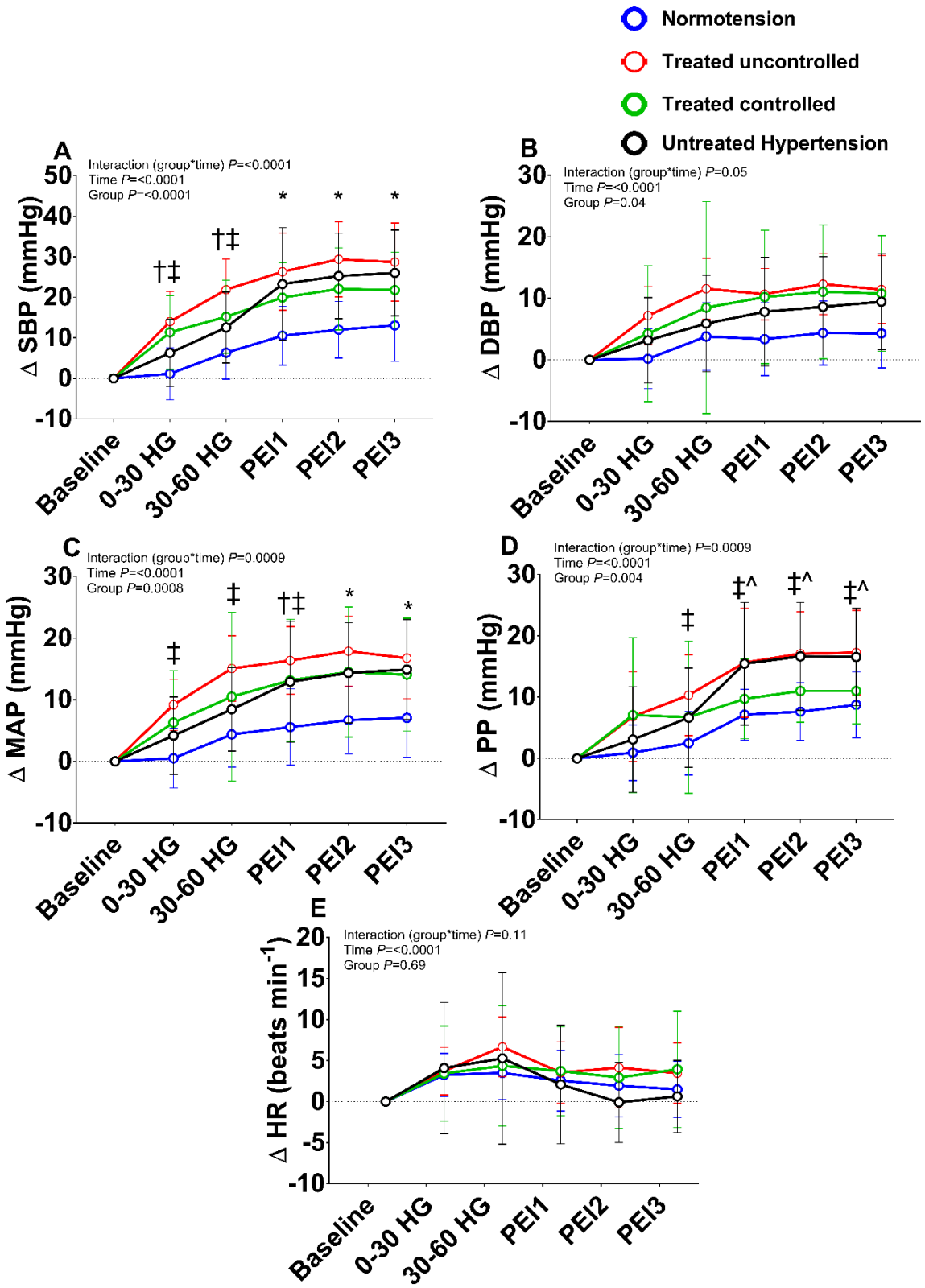


Figure 3-10 The change in haemodynamics from baseline during handgrip testing.

The absolute change from baseline in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) Pulse pressure, and heart rate (HR) during 0-30 and 30-60s isometric handgrip exercise (30% maximal voluntary contraction) and during post-exercise ischemia (PEI) (30 second ± periods PEI1, 2 and 3). * *P*=<0.05 for all groups for normotension. † *P*=<0.05 treated-uncontrolled hypertension vs. normotension. ‡ *P*=<0.05 treated-controlled hypertension vs. normotension. § *P*=<0.05 treated-controlled and treated-uncontrolled hypertension vs. untreated hypertension. # *P*=<0.05 treated-controlled hypertension vs. treated-uncontrolled hypertension. + *P*=<0.05 treated-uncontrolled hypertension vs. untreated hypertension. £ P=<0.05 treated-controlled hypertension vs. untreated hypertension. ^ *P*=<0.05 untreated hypertension vs. normotension. Normotension (blue line), untreated hypertension (black line), treated-uncontrolled hypertension (red line) and treated-controlled hypertension (green line).

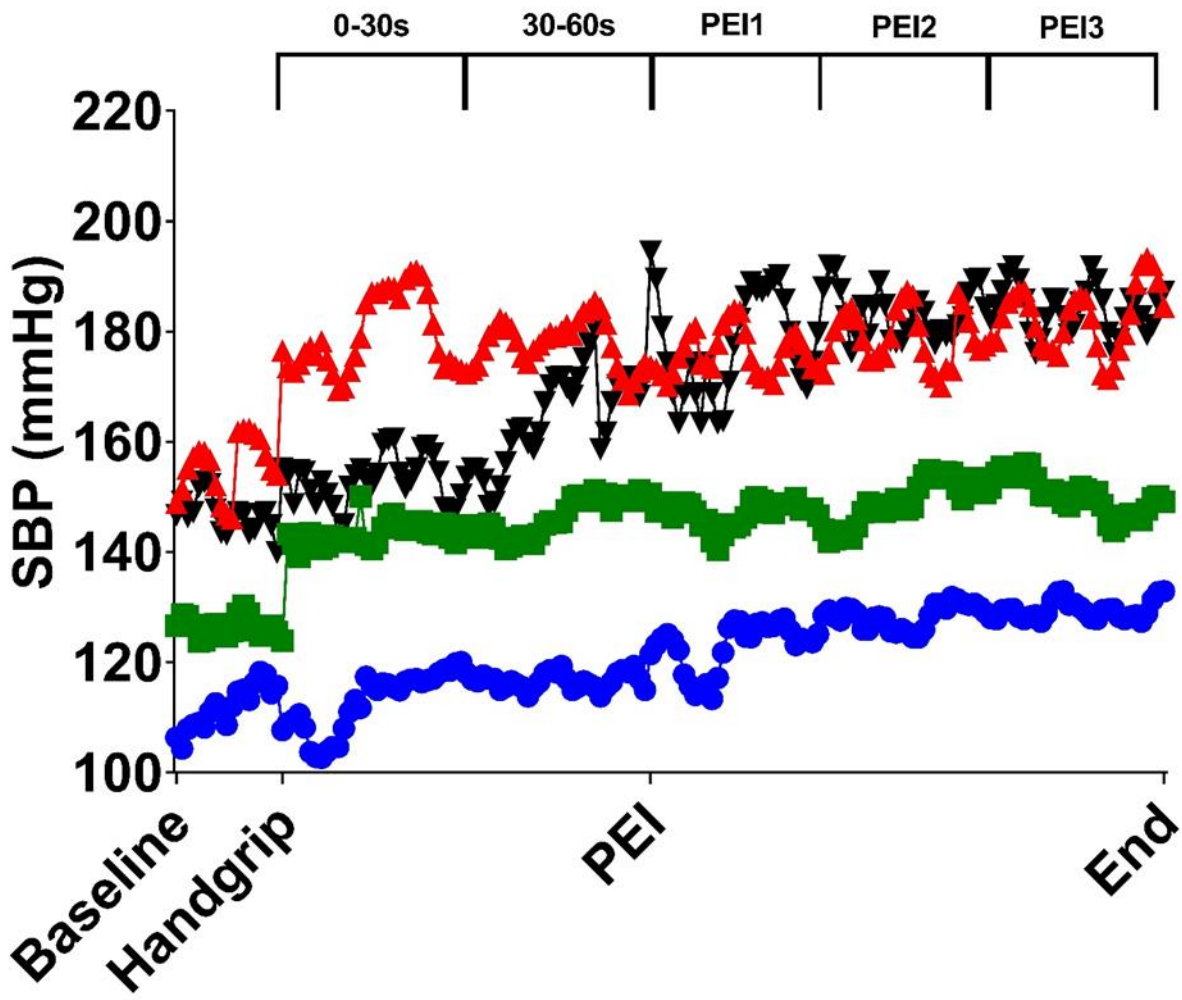


Figure 3-11 Example systolic blood pressure (SBP) responses to isometric handgrip exercise and all of post-exercise ischemia (PEI) in one patient with treated-controlled hypertension (green line), treated-uncontrolled hypertension (red line), untreated hypertension (black line) and normotension (blue line).

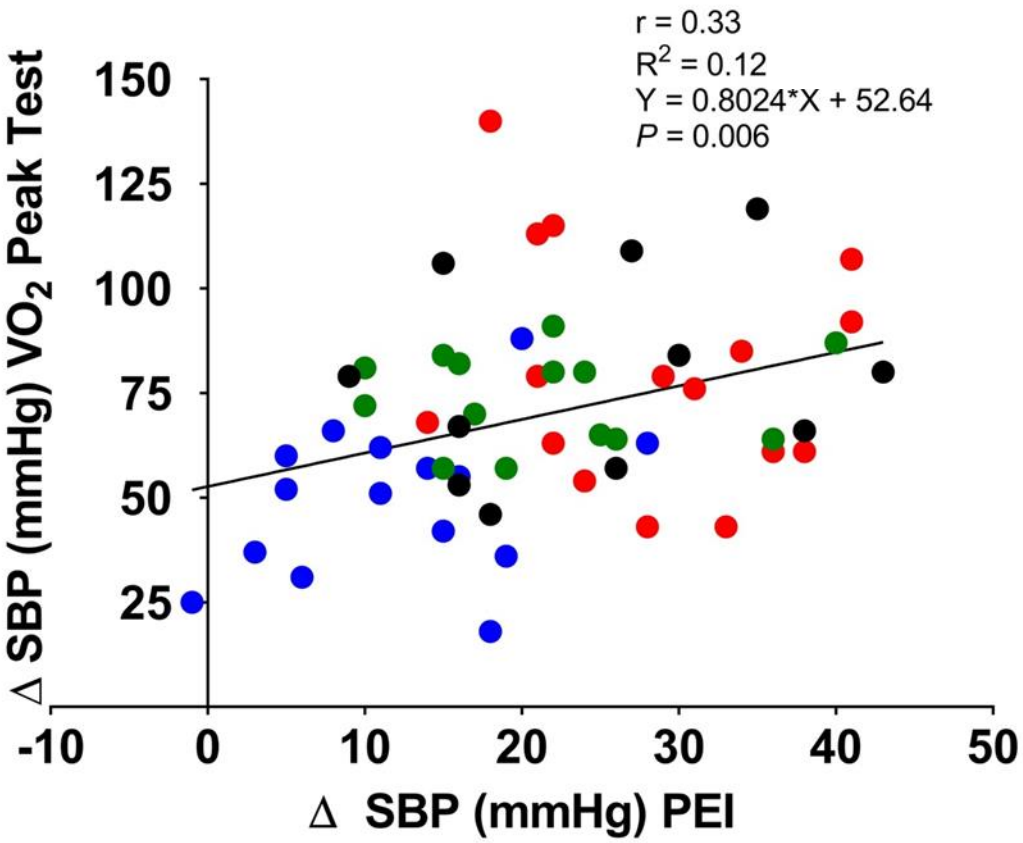


Figure 3-12 Relationship between the change in systolic blood pressure (∆SBP) at peak $\dot{V}O2$ and the change in SBP during post-exercise ischemia (PEI). The data show that increased ∆SBP during the 1 minute 30 seconds of metaboreflex isolation (PEI) is linked to an exaggerated ∆SBP during $\dot{V}O_2$ peak testing. Blue dots, normotension; green dots, treated-controlled hypertension; red dots, treated-uncontrolled hypertension and black dots, untreated hypertension.

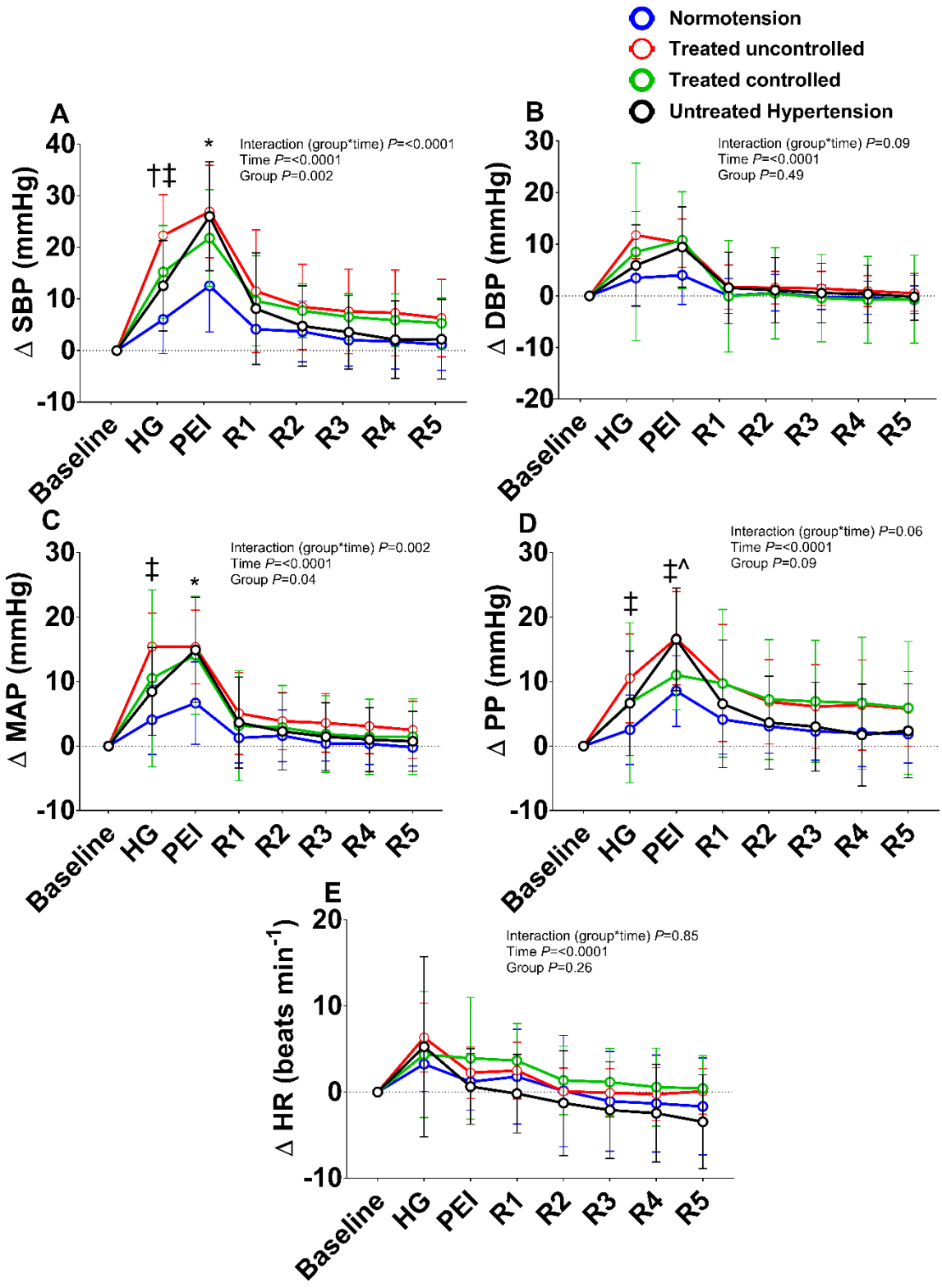


Figure 3-13 The change in haemodynamics during recovery from metaboreflex. The absolute change from baseline in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) Pulse

pressure, and heart rate (HR) during 5 epochs of recovery from metaboreflex testing (R1,R2,R3,R4 and R5). * *P*=<0.05 for all groups for normotension. † *P*=<0.05 treated-uncontrolled hypertension vs. normotension. ‡ *P*=<0.05 treated-controlled hypertension vs. normotension. § *P*=<0.05 treated-controlled and treated-uncontrolled hypertension vs. untreated hypertension. # *P*=<0.05 treated-controlled hypertension vs. treated-uncontrolled hypertension. + *P*=<0.05 treated-uncontrolled hypertension vs. untreated hypertension. £ P=<0.05 treated-controlled hypertension vs. untreated hypertension. ^ *P*=<0.05 untreated hypertension vs. normotension. Normotension (blue line), untreated hypertension (black line), treated-uncontrolled hypertension (red line) and treated-controlled hypertension (green line).

Chapter 4 Arterial stiffness and exaggerated blood pressure responses to exercise in humans with hypertension

4.1 Introduction

During an acute bout of dynamic or isometric exercise, untreated hypertensive individuals have an exaggerated rise in systolic blood pressure (SBP) when compared to normotensive individuals (Delaney et al., 2010, Sausen et al., 2009, Aoki et al., 1983, Chant et al., 2018). The cardiovascular (CV) response to isolation of the metaboreflex component of the exercise pressor reflex is also overactive patients with untreated hypertension (Chant et al., 2018, Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014). The excessive CV response to metaboreflex isolation is partially mediated by the sympathetic nervous system (SNS), as assessed by microneurography (Delaney et al., 2010). Worryingly, an exaggerated increase in blood pressure (BP) (measured in the brachial artery) during an acute bout of dynamic exercise is an independent risk for any type of CV event, end organ damage and mortality (Kurl et al., 2001, Laukkanen et al., 2006), In the study in Chapter 3, the findings indicated that despite adequate control of resting SBP, a group with treated controlled hypertension had an augmented BP response to incremental exercise testing to peak oxygen consumption ($\dot{V}O_2$ peak) as well as isolation of the metaboreflex (post-exercise ischemia (PEI)) compared to normotensive individuals. However, it is unlikely that the metaboreflex alone mediates the exaggerated BP response to exercise in patients with hypertension. Other potential candidates for an exaggerated rise in BP during exercise in patients with treated controlled hypertension include increased large artery (e.g. aorta, brachial, carotid artery) stiffness and abnormal central haemodynamics.

Pulse wave velocity is the gold standard measurement of aortic stiffness (Weber et al., 2015, Van Bortel et al., 2012). Resting pulse wave velocity predicts adverse CV events in the general population (Willum-Hansen et al., 2006, Mattace-Raso et al., 2006). Pulse wave velocity is elevated and remains predictive in treated and untreated hypertensive patients (Pereira et al., 2013, Laurent et al., 2001, Laurent et al., 2006, Laurent et al., 2003) independent of other risk factors such as previous CV disease, resting SBP, diabetes and age. Elevated large artery stiffness in patients with essential hypertension may decrease the ability of the blood vessels to distend when they face exercise induced elevations in CO (Schultz and Sharman, 2014). Tsioufis et al. (2008) found that untreated hypertensives with an exaggerated rise in peripheral SBP during maximal treadmill testing had higher resting carotid-femoral pulse wave velocity when compared to untreated hypertensives with a normal peripheral BP response to exercise. An elevated carotid-femoral pulse wave velocity has also been shown to be positively related to SBP during submaximal treadmill exercise testing in treated and untreated hypertensive individuals (Thanassoulis et al., 2012). This study included patients with diabetes and high cholesterol (hypercholesterolemia), as well as obese individuals (Thanassoulis et al., 2012). All of these CV risk factors are known to alter the CV response to exercise and therefore the effects of arterial stiffness on the BP response to exercise remains unclear (Thanassoulis et al., 2012, Sharman et al., 2007).

Pulse wave velocity is the speed at which a pressure wave is transmitted down the arterial tree. Upon leaving the left ventricle blood generates a forward pressure wave that expands the aorta and shunts blood forward. The forward wave generated by the left ventricle is returned (via a process called reflection) late during diastole in young healthy individuals due to compliant large arteries (Hirata et al., 2006). Wave reflection does not influence aortic BP in young healthy individuals and the brachial artery BP is higher than aortic BP (Hirata et al., 2006). This leads to elevated brachial BP when compared to aortic BP in young individuals (Schultz et al., 2012). However, ageing is associated with increased large artery stiffness and the pressure wave is returned during systole (McEniery et al., 2014, Lee and Oh, 2010, McEniery et al., 2005). In the elderly, wave reflection early in systole causes aortic BP to rise to a similar extent as peripheral BP. Of concern, in older hypertensive individuals' arterial stiffness is further increased and the wave reflection augments aortic BP higher (Hirata et al., 2006, McEniery et al., 2014, Fantin et al., 2007). This causes aortic BP to rise to a similar level as peripheral brachial BP, with brachial SBP being similar to aortic SBP (Hirata et al., 2006, Fantin et al., 2007). The heart, brain and the kidney are exposed to the BP generated by the left ventricle, rather than the brachial artery. Importantly, resting aortic BP is an independent risk factor for end-organ damage, adverse CV events and total mortality when compared to BP's measured in the brachial artery (Vlachopoulos et al., 2010, Kostapanos et al., 2016). An adaptation of the Windkessel model (Wang et al., 2003) has also been used in an attempt to describe differences in aortic and brachial artery BPs. A portion of the stroke volume (~40%) generated during systole is stored in the vessel wall as blood moves into the aorta quicker than it can leave. The

distention of the aorta by a rise in volume of blood causes an increase in pressure (Windkessel pressure). This Windkessel function acts as a reservoir for blood during systole (Davies et al., 2010). In addition, loss of compliance of the aorta has been shown to cause an elevated aortic pressure for a given volume of blood (Davies et al., 2010). The adapted Windkessel model account for both the Windkessel reservoir function and wave reflection (Wang et al., 2003). This model has shown that forward waves are more important than reflected waves at rest and during exercise (Wang et al., 2003, Davies et al., 2010, Schultz et al., 2013a). An interpretation could be that increased resting aortic BP in hypertensive individuals, associated with elevated aortic stiffness (Pereira et al., 2013, Laurent et al., 2001, Laurent et al., 2006, Laurent et al., 2003) would lead to a reduced capacity of the aorta to store potential energy during exercise induced increases in stroke volume. This would lead to an elevated forward pressure from the left ventricle and an increase in peripheral (brachial) BP. Importantly, resting aortic BP is related to submaximal intensity treadmill exercise brachial SBP in male and female hypertensive patients (Thanassoulis et al., 2012).

Different antihypertensive medications that cause a similar lowering of brachial artery BP have markedly different effects on pulse wave velocity and aortic BP (Blacher et al., 2005, Boutouyrie et al., 2011, Protogerou et al., 2009). In patients with hypertension, treated or untreated, it is unknown whether increased aortic BP and increased pulse wave velocity increase the brachial artery BP response to submaximal and peak dynamic exercise. This is important because adequately reducing aortic BP and pulse wave velocity in patients with hypertension could be

an effective way of controlling BP in the brachial artery during exercise. This would have a therapeutic benefit to the hypertensive patient and reduce the CV risks associated with exercise in this group.

The aims of this study were too firstly assess whether resting aortic BP and pulse wave velocity are related a change in peripheral SBP during submaximal and maximal exercise testing ($\dot{V}O_2$ peak test). The secondary aim was to assess what variable was the leading predictor (e.g. age, arterial stiffness and metaboreflex hyperreflexia) of submaximal and peak exercise peripheral SBP. This was completed using multiple linear regression. It was hypothesised that aortic BP and pulse wave velocity would be different among treated controlled, treated uncontrolled, untreated hypertensive and normotensive participants. Secondly, it was hypothesised that aortic BP and pulse wave velocity would be related to the peripheral SBP during submaximal and peak exercise testing. Finally, it was hypothesised that the leading predictors of an exaggerated peripheral SBP response to submaximal and peak exercise will have a different influence on the SBP response to exercise.

4.2 Methods

4.2.1 Participants

The same group of participants were used in this study as the study in Chapter 3. 16 normotensives, 14 treated-controlled, 16 treated-uncontrolled and 11 untreated hypertensives who were matched for age, body mass index (BMI), and for CV fitness ($\dot{V}O_2$ peak) were used for the study in this Chapter (Chapter 3,

Table 3.1 and Table 3.2, pages 190 to 192). All individuals were free from diabetes mellitus (urine dipstick test and self-reported), and were non-obese (Chapter 3, Table 3.1 and Table 3.2, pages 190 to 192). Ethical approval for this study was granted by the Southwest-Exeter NHS REC (16/SW/0004). The information regarding recruitment and inclusion/exclusion criteria for this study can be found in Chapter 3 (Chapter 3; section 3.2, page 151).

4.2.2 Study Design

This study was a case-control study and the researcher was blinded to the participant BP classification until the data analysis was complete. Participants visited the laboratory on 2 separate occasions.

4.2.3 Screening procedures

The screening procedures used for Chapter 4 were the same as Chapter 3 (Chapter 3; section 3.2.3 Screening procedures, page 153). A flow diagram for the initial screening can be seen in Figure 3.2. (Chapter 3, page 197). At the end of the initial screening visit, the participants performed a $\dot{V}O_2$ peak test on a cycle ergometer (Chapter 2; section 2.3.1, page 94 and Chapter 3; section 3.2.1, page 151). See Chapter 3 (section 3.2.1, page 151) for the criteria used to define $\dot{V}O_2$ peak.

4.2.4 Study visit design

Participants returned at a similar time of day for a follow up visit to the screening visit with at least 48 hours between. Firstly, the participants clinic BP was assessed using the same protocol outlined in Chapter 3 (section 3.2.3, page 153). Following this, participants were asked to rest for 10 minutes prior to the measurement of pulse wave analysis and pulse wave velocity.

4.2.5 Arterial Stiffness

4.2.5.1 Pulse Wave Analysis

Pulse wave analysis was used to measure aortic BP. For a more detailed description of pulse wave analysis, please see Chapter 2, section 2.4. (page 99). Applanation tonometry was performed using SphygomoCor (SpygmoCor System, AtCor Medical, Sydney) on the right radial artery whilst the participant lay supine. Firstly, the participants resting BP was measured in the supine position from the brachial artery. In accordance with the European Society of Hypertension guidelines resting BP was assessed using an automatic oscillometric monitor (Omron, 705IT, Omron Healthcare Europe) (Chapter 2, section 2.4, page 99). These readings were then entered into the SpygmoCor System (AtCor Medical, Sydney). To perform this analysis, a tonometer was placed over the radial artery for a sufficient period of time so that a radial pulse trace could be recorded. The SphygomoCor (SpygmoCor System, AtCor Medical, Sydney) system has an inbuilt calculation that assesses the variability of the recording, this is called the operator index and is a scale of 0-100 (100 being the best quality). If the operator index was <= 95 a repeat reading was done until the score was > 95. Central

pressures were estimated using an inbuilt transfer function in the SphygmoCor system, which estimates the central BP waveforms from the radial pulse waveform (SpygmoCor System, AtCor Medical, Sydney) (Karamanoglu et al., 1993, Chen et al., 1997). From the recreated central pressure waveform, the software calculates aortic augmentation pressure (which is defined as the difference between the peak 1 and peak 2 wave in the central pressure waveform), aortic SBP, aortic PP and augmentation index (AIx %) (aortic augmentation pressure/aortic PP * 100) (Figure 4.1, page 249). The AIx % is taken as a marker of wave reflection (Hirata et al., 2006) (Figure 4.1, page 249). AIx is also reported as AIx 75 which is normalised to a heart rate (HR) of 75, which allows the comparison between individuals with different resting HR. Pulse wave analysis was performed two times in each participant and the mean value is presented in the study in this Chapter. Following the measurement of pulse wave analysis, the participant was asked to rest for 5 minutes prior to the assessment of pulse wave velocity.

4.2.5.2 Pulse Wave Velocity

Aortic stiffness was assessed non-invasively using pulse wave velocity in both hypertensives and normotensive participants (see Chapter 2, section 2.4.1.1, page 100). See Chapter 2 for repeatability data for pulse wave velocity (section 2.5.2. Table 2.4, page 128 and Figure 2.8, page 141). Pulse wave velocity was measured via arterial tonometry (SpygmoCor System, AtCor Medical, Sydney) between the carotid-femoral arteries as this is the most valid method for assessing large arterial stiffness over carotid-brachial arteries (Tillin et al., 2007) (see Chapter 2, section

2.4.1, page 99). The distance between the sternal notch and the carotid and femoral artery was carefully measured and entered into the SpygmoCor System (AtCor Medical, Sydney) in mm. Following this the carotid and the femoral waveform were captured respectively. Pulse wave velocity was calculated automatically by the SpygmoCor (SpygmoCor System, AtCor Medical, Sydney) using the equation:

$$\text{Pulse Wave Velocity} = d_{PWV}/\Delta t \quad \text{[equation 4.1]}$$

d_{PWV} = distance between sternal notch to femoral artery (cm) – distance between sternal notch carotid artery (cm)

Δt = the change in time

4.2.6 Handgrip and Metaboreflex testing

See Chapters 2 and 3 for the protocol used for isometric handgrip exercise and isolation of the metaboreflex (section 2.6, page 108 and section 3.2.4.1, page 155).

4.2.7 Physiological Monitoring During $\dot{V}O_2$ Peak and Metaboreflex Testing

4.2.7.1 $\dot{V}O_2$ peak test

The methods used for physiological assessment during the $\dot{V}O_2$ peak test can be found in Chapter 3 (section 3.2.3.1, page 154).

4.2.7.2 Handgrip and Metaboreflex isolation

The methods used for physiological assessment during handgrip, exercise ischemia and metaboreflex isolation (post-exercise ischemia (PEI)) can be found in Chapter 3 (section 3.2.4.1, page 155).

4.2.8 Power calculations

This Chapter was part of a larger study (Chapter 3) and the power calculations were based on the SBP response to a maximal exercise test (this was the primary outcome) rather than resting aortic BPs and pulse wave velocity. The power calculations can be found in Chapter 3 (section 3.2.6, page 156).

4.2.9 Data analysis

4.2.9.1 $\dot{V}O_2$ peak test

The methods used to analyse the $\dot{V}O_2$ peak test are described in Chapter 3 (section 3.2.7.1, page 157).

4.2.9.2 Metaboreflex testing

The methods used to analyse isometric handgrip exercise, exercise ischemia and PEI are described in Chapter 3 (section 3.2.7.2, page 158).

4.2.10 Statistical analysis

To avoid repetition the statistical analysis performed to assess for differences in the baseline, $\dot{V}O_2$ peak test, isometric handgrip exercise, exercise ischemia and PEI are not described again here and can be found in Chapter 3 (section 3.2.9, page 162).

The group averages (normotensives, untreated hypertensives, treated-controlled hypertensives and treated-uncontrolled hypertensives) for differences in resting aortic augmentation pressure, aortic SBP, aortic PP, AIx, AIx 75 and pulse wave velocity were compared using an ordinary 1-way analysis of variance (ANOVA), with a Tukey test for multiple comparisons. Data were checked for normality using a D'Agostino-Pearson normality test. To examine whether there was a relationship between pulse wave velocity, aortic SBP, AIx, AIx 75 and the absolute change in SBP metaboreflex isolation (PEI) and the absolute change in SBP during VO_2 peak testing, a Pearson's correlation coefficient was also performed to assess relationships with the change in peripheral SBP during submaximal intensity (51-75% of $\dot{V}O_2$ peak) and peak exercise. Forced entry multiple linear regression was then performed (SPSS Statistics 24; IBM Corp, Armonk, New York) to make a model which predicts the individual contribution of the independent variables to the change in SBP during submaximal intensity (51-75% of $\dot{V}O_2$ peak) and peak exercise.

Data analysis and statistical analysis were completed using LabChart 7 (AD Instruments), Spike 2 (Cambridge Electronic Designs), Microsoft Excel (Microsoft

Corp., Redmond, WA), IBM SPSS Statistics 24 (IBM Corp, Armonk, New York), R studio version 3.4.1 (RStudio: Integrated Development Environment for R, Boston, MA) and GraphPad version 7 (GraphPad Software, La Jolla California USA). Data are reported as mean ± standard deviation (SD) The α-level was set at 0.05.

4.3 Results

4.3.1 Participant demographics

The baseline participant demographics for normotensive, treated uncontrolled, treated controlled and untreated hypertensive participants can be found in Table 4.1 (page 243).

4.3.2 $\dot{V}O_2$ Peak Test and Metaboreflex testing

The changes in haemodynamics during $\dot{V}O_2$ peak testing, isometric handgrip, exercise ischemia and PEI can be found in Chapter 3 (section 3.3.2 and 3.3.3 and Figure 3.5-3.13).

4.3.3 Pulse wave analysis

All measures that were assessed from arterial tonometry can be found in Figure 4.2 (page 250) and Table 4.1 (page 243). There was a difference between groups for aortic SBP ($F(3,53) = 7.603$; $P=0.0003$; $\eta^2 = 0.3$). Treated-uncontrolled (138±17 mmHg) and untreated hypertensive participants (134±20 mmHg) had elevated aortic SBP when compared to normotensive (113±10 mmHg) participants ($P=0.0002$ and $P=0.007$ respectively; Tukey test). There were no

differences in aortic SBP between treated controlled (127±17 mmHg) and normotensive (113±10 mmHg) (*P*=0.09) participants. No differences were found between treated controlled and treated uncontrolled (*P*=0.20) or untreated hypertensive (*P*=0.66) participants in aortic SBP. In addition, there were no differences in aortic SBP between patients with treated uncontrolled and untreated hypertension (*P*=0.91). A difference was found between groups for aortic DBP ($F(3,53) = 5.091$; *P*=0.004; $\eta^2 = 0.22$). More specifically, untreated (88±11) and treated uncontrolled (88±11) hypertensive groups had a larger aortic DBP compared to normotensives (77±6) (*P*=0.03 and *P*=0.01 respectively). Treated controlled hypertensives (79±10) had a similar aortic DBP when compared to normotensives (77±6) (*P*=0.89). Treated controlled hypertensive participants had a comparable aortic DBP compared to patients with treated uncontrolled and untreated hypertension (*P*=0.08 and *P*=0.16 respectively, Tukey test). Finally, no difference was found for aortic DBP between participants with treated uncontrolled hypertension and untreated hypertension (*P*=0.99). There was also a difference in aortic PP between groups ($F(3,53)=4.743$; *P*=0.005; $\eta^2 = 0.21$). Interestingly treated controlled (48±9 mmHg) and treated uncontrolled hypertensives (50±13 mmHg) had an elevated aortic PP compared to normotensives (36±8 mmHg) (*P*=0.04 and *P*=0.004 respectively). There was no difference in aortic PP between untreated hypertensives (46±15) compared to normotensives (36±8) (*P*=0.10). Again, no differences were found between treated and untreated hypertensive individuals (*P*=>0.05).

There were no differences in the aortic augmentation pressure ($F(3,53) = 2,284$; *P*=0.09, $\eta^2 = 0.11$), AIx (%) ($F(3,51)=0.2501$; *P*=0.86, $\eta^2 = 0.001$) or the AIx 75

(F(3,50)=0.028;*P*=0.99, η^2 = 0.001) between the groups (Figure 4.2, page 250 and Table 4.1, page 243). No differences were found for the amplification between aortic SBP and the brachial SBP between the groups (F(3,53) = 0.61; *P*=0.61; η^2 = 0.11). In addition there was no difference for the amplification of the aortic DBP and brachial DBP between the groups (F(3,53) = 0.61; *P*=0.61; η^2 = 0.03). Finally, no difference was found for the PP amplification (brachial PP – aortic PP) between the groups (F(3,53) = 2.16; *P*=0.10; η^2 = 0.11). There was a positive correlation between aortic SBP and daytime ambulatory SBP (r = 0.53; R^2 = 0.27; *P*=<0.0001; Figure 4.3 for linear regressions, page 251). Similarly, a positive correlation was also found between aortic DBP and daytime ambulatory DBP (r = 0.64; R^2 = 0.42; *P*=<0.0001; Figure 4.3 for linear regressions, page 251). A comparable positive correlation was found for aortic PP and brachial PP (r = 0.49; R^2 = 0.21; *P*=0.0001; Figure 4.3 for linear regressions, page 251).

4.3.4 Pulse wave velocity

There was a difference found between the groups for pulse wave velocity (m/s) (F(3,53)=3.062; *P*=0.04, η^2 = 0.13). Untreated hypertensives (11.2±2 m/s) had an expected elevated pulse wave velocity score when compared to normotensives (8.8±1.5 m/s) (*P*=0.048). There were no differences in pulse wave velocity between participants with treated controlled (10.6±2.3 m/s) or treated uncontrolled (10.6±2.5 m/s) hypertension when compared to individuals with normotension (8.8±1.5 m/s; *P*=0.14 and *P*=0.11 respectively). In addition, there were no difference between treated uncontrolled, treated controlled and untreated groups with hypertension (all *P*=>0.05).

4.3.5 The effect of arterial stiffness on the change in SBP during submaximal and peak $\dot{V}O_2$ peak testing

4.3.5.1 Submaximal intensity (50-75% $\dot{V}O_2$ peak testing)

There was a positive correlation for both aortic PP and SBP against the change in brachial SBP during submaximal intensity exercise (50-75% $\dot{V}O_2$ peak testing) ($r = 0.28$; $R^2 = 0.08$; $P=0.03$ and $r = 0.27$; $R^2 = 0.08$; $P=0.04$ respectively; Figure 4.4 for linear regressions, page 253).

There was no correlation between augmentation pressure (mmHg), AIx (%) or the AIx 75 and the change in brachial SBP that occurred during submaximal intensity (50-75%) $\dot{V}O_2$ peak testing (Figure 4.4, page 253). There was no correlation between pulse wave velocity (m/s) at rest and the change in brachial SBP during submaximal intensity (50-75%) dynamic exercise ($r = 0.19$; $R^2 = 0.04$; $P=0.10$; Figure 4.4 for linear regressions, page 253).

4.3.5.2 Peak $\dot{V}O_2$ peak testing

Unlike submaximal exercise (50-75% $\dot{V}O_2$ peak), there was a positive correlation between resting pulse wave velocity and the peak change in brachial SBP (mmHg) during $\dot{V}O_2$ peak testing ($r = 0.29$; $R^2 = 0.08$; $P=0.03$; Figure 4.5, page 254). In addition, aortic PP (mmHg) and aortic SBP (mmHg) were also both positively correlated to the maximal change in brachial SBP during $\dot{V}O_2$ peak testing ($r = 0.37$; $R^2 = 0.14$; $P=0.004$ and $r = 0.43$; $R^2 = 0.18$; $P=0.0009$ respectively). There was no correlation between augmentation pressure (mmHg),

AIx (%) and AIx 75 and the change in brachial SBP at maximal $\dot{V}O_2$ peak testing (Figure 4.5, page 254).

4.3.6 Multiple regression analysis

The study in Chapter 3 showed that there was a positive correlation between the change in brachial SBP measured during metaboreflex isolation (PEI) and the change in brachial SBP at peak exercise ($\dot{V}O_2$ peak testing) (r = 0.36; R2 = 0.13; *P*=0.007; section 3.3.3.6; Figure 3,12, page 213). To assess whether metaboreflex hyperreflexia or increased arterial stiffness was a larger contributor towards an exaggerated change in SBP during peak and submaximal $\dot{V}O_2$ peak testing a forced entry multiple linear regression test was performed. The independent variables chosen (aortic PP, pulse wave velocity, age, daytime ambulatory SBP and the change in SBP during PEI (metaboreflex isolation) (Delaney et al., 2010, Sausen et al., 2009, Tsioufis et al., 2008, Thanassoulis et al., 2012) were based on previous research that has suggested that these variables increase the risk of having an exaggerated rise in brachial SBP during submaximal and peak exercise.

4.3.6.1 Submaximal intensity (50-75% $\dot{V}O_2$ peak testing)

No multicollinearity was found between independent variables (Table 4.2 and 4.3, page 245 and 246). More specifically, all of the calculated variance inflation factors were below 10 and the tolerance levels were all above 0.2 (Table 4.2 and 4.3, page 245 and 246). A significant regression equation was found (F(5,46)=2.859; *P*=0.025), R^2 = 0.237). The change in brachial SBP during submaximal intensity (50-75%) $\dot{V}O_2$ peak testing was predicted only by the change in brachial SBP

during PEI (37.363+0.409; *P*=0.005, Table 4.3, page 246). None of the other independent variables were significantly related to the change in SBP during submaximal exercise (50-75% $\dot{V}O_2$ peak, Table 4.3, page 246). The augmentation in SBP during submaximal intensity (50-75%) $\dot{V}O_2$ peak testing increased 0.409 mmHg per 1 mmHg change in peripheral SBP during PEI (metaboreflex isolation).

4.3.6.2 Peak $\dot{V}O_2$ peak testing

A second forced entry multiple linear regression was also performed to predict the change in brachial SBP during peak exercise using the same independent variables. No multicollinearity was found between independent variables (Table 4.4 and 4.5, pages 247 and 248). More precisely, all of the calculated variance inflation factors were below 10 and the tolerance levels were all above 0.2 for all of the independent variable interactions (Table 4.4 and 4.5, page 247 and 248). A significant regression was found (F(5,46) = 4.332, *P*=0.003), with an R^2 = 0.246. The predicted change in peak brachial SBP during a $\dot{V}O_2$ peak test was equal to 45.017 + 0.594 (PEI; *P*=0.047) + 0.578 (aortic PP; *P*=0.048) (Table 4.5, page 248). Participants peak change in brachial SBP during a $\dot{V}O_2$ peak test increased by 0.594 mmHg for each mmHg increase in brachial SBP during PEI (metaboreflex isolation) and 0.578 mmHg for each mmHg increase in aortic PP at baseline. Both the change in brachial SBP (mmHg) during PEI (*P*=0.047) and the resting aortic PP (mmHg) (*P*=0.048) were significant predictors of the peak change in SBP during a $\dot{V}O_2$ peak test. The remaining independent variables were not significant predictors of the absolute change in SBP during peak exercise (Table 4.4, page 247).

4.4 Discussion

The main finding of this study was that elevated aortic PP and metaboreflex hyperreflexia were the most powerful predictors of an exaggerated rise in SBP during maximal exercise ($\dot{V}O_2$ peak test), even when accounting for other factors that are known to mediate an exaggerated peripheral BP response to exercise. In addition, metaboreflex hyperreflexia was the only significant predictor of the augmentation of SBP during submaximal exercise (50-75%) during $\dot{V}O_2$ peak testing. Finally, the study indicates that aortic PP remains elevated in patients with treated controlled hypertension. However, pulse wave velocity was only elevated in untreated hypertensive individuals.

4.4.1 Pulse wave analysis and pulse wave velocity

Current clinical guidelines do not aim at lowering aortic BP alongside brachial BP, perhaps due to difficulties with its measurement during a patient visit to their clinician or general practitioner. This is concerning considering aortic BP is the pressure that the heart, the kidney and the arteries supplying the brain are exposed to (Hirata et al., 2006). Indeed, elevated aortic PP and aortic SBP are stronger predictors of adverse CV events when compared to brachial PPs and SBPs (Safar et al., 2002, Roman et al., 2007, Pini et al., 2008, Roman et al., 2009, Wang et al., 2009). Although aortic BP has been shown to be a useful marker for adverse CV events, a large-scale randomised trial has not yet been commenced with the end goal of lowering central BP or arterial stiffness. However, a randomised controlled trial found that using aortic BP as a marker of the effectiveness of antihypertensive therapy led to the use of less medications to achieve adequate BP control when

compared to using brachial artery BP measurements in 286 hypertensive patients (Sharman et al., 2013). Most concerning is that both treated controlled and treated uncontrolled hypertensives had elevated aortic PP in this study when compared to normotensive participants. The current view is that drugs that interfere with the renin-angiotensin-aldosterone system (RAAS), such as angiotensin converting enzyme inhibitors (ACEi) are more effective at lowering aortic pressures when compared to other anti-hypertensive medications (Boutouyrie et al., 2011). This may be due to the profibrotic effect of angiotensin II on the arterial wall (Laurent et al., 2005). It is interesting to note that in the present study a large proportion of the treated hypertensives were taking ACEi (44% of treated uncontrolled hypertension and 88% of treated controlled hypertension; Chapter 3; Table 3.1, page 190) and angiotensin receptor blockers (25% of treated uncontrolled hypertension and 31% of treated controlled hypertension (Chapter 3; Table 3.1, page 190) yet still had elevated aortic PP. Therefore, general practitioners and clinicians may consider measurements of aortic PP when seeing hypertensive patients.

It remains to be seen why aortic SBP and DBP were not elevated in treated controlled hypertensives compared to normotensive participants, whereas central PP was different. However, there was a large effect size ($\eta = 0.3$) calculated for aortic SBP. This would suggest that with a larger sample size differences between treated controlled hypertension (127±17) and normotension (113±10) may have been found. Similarly, a large effect size was also calculated for the effect of group on aortic DBP ($\eta = 0.22$).

Only untreated hypertensive individuals had elevated pulse wave velocity in this study when compared to normotensive individuals. It is unclear why the arterial tonometry used to calculate aortic PP and pulse wave velocity measurement was inconsistent with each other as they both indirectly measure arterial stiffness. One study found that reductions in pulse wave velocity were associated also with reductions in aortic SBP and PP (Ait-Oufella et al., 2010). This study was powered to assess the differences in SBP during maximal exercise, and more participants are likely to be needed to see differences in pulse wave velocity. Similar to aortic SBP and DBP a large effect size was calculated for pulse wave velocity ($\eta = 0.22$). This may suggest differences may have been found between the groups if this study had been powered to find differences in pulse wave velocity.

4.4.2 Identifying the cause of an exaggerated SBP response to $\dot{V}O_2$ peak testing

The exact mechanism that mediates exaggerated changes in SBP during exercise is unknown. In the study in Chapter 3 it was shown that metaboreflex hyperreflexia in treated and untreated hypertension partially mediates an exaggerated change in SBP during submaximal intensity (51-75% $\dot{V}O_2$ peak) and peak exercise testing ($\dot{V}O_2$ peak test). It is well documented that metaboreflex hyperreflexia is present in people with untreated hypertension (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014, Chant et al., 2018). An additional mechanism that mediates an exaggerated rise in peripheral SBP during exercise in individuals with essential hypertension is increased pulse wave velocity (Tsioufis et al., 2008). In the Framingham study it was shown that both pulse wave velocity and aortic PP were positively associated with an increased brachial SBP during a sub-maximal

exercise test on a treadmill (Thanassoulis et al., 2012). In the current study, prior to adjustment for known mediators of an exaggerated brachial SBP response to exercise pulse wave velocity, aortic PP and the change in peripheral SBP during PEI (metaboreflex isolation) were positively correlated to an exaggerated change in brachial SBP at peak exercise, which would support the Framingham study (Tsioufis et al., 2008). However, after accounting for age, daytime ambulatory SBP, pulse wave velocity, the change in brachial SBP during PEI (metaboreflex isolation) and aortic PP (independent variables), the strongest predictors of an exaggerated change in absolute SBP during maximal exercise were both central aortic PP and the change in SBP during PEI (metaboreflex isolation). Together, aortic PP and the absolute change in SBP during PEI accounted for 25% (R^2=0.25) of the change in SBP at peak exercise. In addition, the change in SBP during sub-maximal exercise was found to be predicted only by the change in SBP during PEI. Metaboreflex hyperreflexia accounted for 18% (R^2=0.15) of the change in SBP during sub-maximal exercise. It is clear that other mechanisms are contributing substantially to the exaggerated SBP response to submaximal and peak exercise in these groups, as only 15% of the submaximal and 25% of the peak change in absolute SBP can be explained by this study. It is likely that other neural mechanisms, such as the mechanoreflex (Velasco et al., 2015, Greaney et al., 2015a), central command (Liang et al., 2016), peripheral chemoreceptors (Pijacka et al., 2018) and impaired aortic and carotid baroceptor function (Brum et al., 2000) are also contributing to the SBP response to exercise. Metabolic irregularities have also been highlighted in mediating an exaggerated SBP response to exercise, such as hypercholesterolemia (Thanassoulis et al., 2012, Sharman et al., 2007) and insulin resistance (Park et al., 2006).

The focus of this Chapter was on the SBP response to exercise due to the well documented relationship between an exaggerated SBP and elevated CV risk (Kurl et al., 2001, Laukkanen et al., 2006). In addition, there is very limited data available regarding the DBP during exercise and CV risk. MSNA is closer related to DBP than SBP (Sundlöf and Wallin, 1978) and MSNA contributes to the exaggerated pressor response to exercise in hypertension (Delaney et al., 2010). Future research will need to assess the DBP response to exercise and elevated CV risk in hypertension. This Chapter supports previous research that highlights that aortic PPs are importantly linked to SBP during dynamic exercise and helps further explain the abnormal pathophysiology during exercise in treated controlled hypertension highlighted in the study in Chapter 3.

4.4.3 Mechanisms

In the simplest form the two-element Windkessel model describes the circulation as a function of resistance and capacitance (total peripheral resistance (TPR) and compliance respectively) (Dart and Kingwell, 2001). Compliance is defined as the capacity of a large artery to accommodate increases in the volume of blood (change in volume/change in pressure) (Dart and Kingwell, 2001). The majority of systemic arterial compliance is determined by the branches of the aorta and the aorta itself (Liu et al., 1986). Aortic compliance decreases at higher pressures and is dependent on the initial volume conditions (Liu et al., 1986). However, an issue with the two-element Windkessel model is that it assumes that all pressure changes occur instantly upon left ventricular contraction and that it does not account for wave reflection (Dart and Kingwell, 2001). In hypertensive patients,

peripheral arterial stiffness is elevated, and the wave reflection occurs earlier in systole and contributes to the aortic BP (Fantin et al., 2007). Another mechanism to explain heightened aortic PP at rest in treated controlled and treated uncontrolled hypertension is the alternative Windkessel model (Wang et al., 2003, Davies et al., 2010). This model focuses on an excess pressure that is calculated from aortic pressure and Windkessel pressure (reservoir pressure = aortic outflow – Windkessel pressure) (Wang et al., 2003). This model states that a forward wave generated by the left ventricle and the Windkessel reservoir function are more important when describing aortic outflow from the windkessel, aortic PP and peripheral PP when compared to wave reflection (AIx) (Wang et al., 2003, Davies et al., 2010). The excess pressure has is the minimum amount of work that the left ventricle has to perform to eject blood into the aorta. The Windkessel reservoir is important in dampening the aortic PP and brachial PP. The elevated aortic PP in the treated controlled and uncontrolled hypertensive groups may have caused an increased Windkessel pressure in the aorta during left ventricular contraction (Wang et al., 2003, Davies et al., 2010, Heffernan et al., 2013). This would cause the aorta to lose its ability to act as a reservoir during systole and a faster forward wave would occur (Wang et al., 2003). Moreover, during diastole there would be less potential energy stored in the aorta and a reduced recoil of the vessel, which would lead to a widening of the aortic PP (Wang et al., 2003). Antihypertensive medications have a mixed effect on aortic BP (Williams et al., 2006) and a less compliant aorta would be less able to expand under increasing volumes of blood (Schultz et al., 2013a, Heffernan et al., 2013). This would have the effect of causing exaggerated increases in brachial artery SBP due to augmented forward waves from the left ventricle and a reduced Windkessel reservoir effect (Schultz et al.,

2013a). In addition, aortic impedance (Zo) is the resistance of the aorta to the left ventricular stroke volume (Wesseling et al., 1993). Upon left ventricular contraction blood is forced into the aorta but the aorta already contains a certain volume of blood and this opposes left ventricular stroke volume (Wesseling et al., 1993). This would contribute to aortic BP because a heightened Zo would mean the left ventricle has to work harder to pump blood into the aorta. Interestingly, Zo has been shown to be elevated in hypertension (Li and Ahn, 2012, Bollache et al., 2015). These factors may help to explain why treated controlled hypertensives have an exaggerated rise in brachial BP during exercise. A less compliant aorta and elevated Zo would likely expand less during exercise induced increases in stroke volume and this would augment forward waves from the left ventricle and brachial BP (Schultz et al., 2013a).

Pulse wave velocity, especially when measured from the carotid-femoral pulse has been shown to be strongly correlated with aortic stiffness when measured during phase contrast magnetic resonance imaging (Hickson et al., 2010). Recent evidence in 1599 patients found that aortic PP was related to CV events whereas carotid-femoral pulse wave velocity was related more to chronic kidney disease, peripheral artery disease, arterial plaques and microalbuminuria (Bai et al., 2018). This suggests that aortic PP may reflect issues with the myocardium whereas carotid-femoral pulse wave velocity may more accurately predict peripheral diseases. However, it is unclear why aortic PP predicted exaggerated changes in absolute SBP during peak exercise in this study and not pulse wave velocity. Further research is needed to assess how aortic PP and pulse wave velocity change during exercise in a larger group of treated and untreated hypertensives

when compared to normotensive controls. Furthermore, the specific contributions of the forward and backward wave have not been established at rest or during exercise in hypertension (Schultz et al., 2013a).

These alterations in central haemodynamics and metaboreflex hyperreflexia help to explain why treated controlled hypertensives remain at increased risk of CV disease, stroke and total mortality compared to normotensive individuals (Almgren et al., 2005, Lawlor et al., 2011, Brown et al., 2013). It could be speculated that drugs that target metaboreflex hyperreflexia whilst also reducing aortic PP would be effective in lowering exercise BP and reducing the CV risk of treated and untreated hypertensive patients.

4.4.4 Study limitations

General study limitations can be found in Chapter 3 (section 3.4.4, page 187). More specifically, we only measured aortic PP at rest and did not measure what would happen to aortic PPs during exercise. Intra-arterial measurements during exercise have shown that in healthy young individuals that PP amplification is further augmented during dynamic exercise (increased brachial artery PP compared to aortic PP) (Rowell et al., 1968, Kroeker and Wood, 1955). Using arterial tonometry, Sharman et al. (2005) confirmed these results non-invasively during upright cycle ergometer exercise. Sharman et al. (2005) found that AIx (a marker of wave reflection) was reduced during exercise in healthy individuals and this was likely mediated by the exercise induced rise in HR and peripheral vasodilation of the arterioles in the active skeletal muscle (functional sympatholysis) (Sharman et al.,

2005). However, aortic PP still increased but significantly less than brachial artery PP (Sharman et al., 2005). Schultz et al. (2013a) found that aortic PP during exercise in healthy adults was mediated by the forward wave and aortic compliance as compared to the reflected wave. In healthy individuals, functional sympatholysis in the active skeletal muscle ensures that wave reflection is minimal (reduced AIx). This serves to protect the aorta from reflected waves and central BP rises during exercise exclusively by pressure waves generated by the left ventricle (Schultz et al., 2013a). In addition, exaggerated increases in SNA during exercise, coupled with impaired functional sympatholysis in the active skeletal muscle would be expected to increase the contribution of the reflected wave leading to elevated aortic PP. This has not directly been assessed in treated or untreated hypertension. Nevertheless, it is known that SNA is exaggerated during exercise in untreated hypertension and that functional sympatholysis is impaired in these individuals (Vongpatanasin et al., 2011, Price et al., 2013, Delaney et al., 2010). Additionally, the data from Chapter 3 suggest that metaboreflex hyperreflexia is augmented in treated hypertensives compared to normotensive controls, which is known to be mediated by the SNS (Delaney et al., 2010). Based on the results of this study and of Chapter 3, future studies should use arterial tonometry during exercise to assess central haemodynamics in treated and untreated hypertension. This could potentially highlight treated controlled hypertensives who are at especially high risk for adverse CV events. This study also only included a small number of participants (n=59), future studies will need to include a larger number of participants to reconfirm these findings in a larger population. Furthermore, due to the low number of participants in each group we were unable to assess the effect of different anti-hypertensive medications on aortic PP at rest and its relationship with exercise

SBP. This is important to assess because studies have shown mixed effects of different anti-hypertensive medications on aortic PP and pulse wave velocity (Blacher et al., 2005, Boutouyrie et al., 2011, Protogerou et al., 2009). Larger multi centre studies will need to assess aortic BP and brachial artery BP during exercise in treated hypertension to assess the variety of different anti-hypertensive medications available.

4.5 Conclusions

This study has highlighted that despite adequate control of baseline brachial artery SBP, treated controlled hypertensives have heightened resting aortic PP. Importantly, this contributes to the exaggerated rise in brachial artery SBP seen in treated controlled hypertension discussed in Chapter 3. Even after adjustment for other known risk factors for an exaggerated SBP response to exercise, aortic PP and metaboreflex hyperreflexia remained positively associated with an exaggerated peripheral SBP response to exercise. This research suggests that central haemodynamic and metaboreflex hyperreflexia should be targeted for optimal control of BP during exercise. Targeting these mechanisms successfully would reduce the associated CV risk of exercise in the hypertensive population.

4.6 Tables

Table 4-1 Participant Characteristics.

Participant demographics	Normotension	Untreated Hypertension	Treated-uncontrolled hypertension	Treated-controlled hypertension
N	16	11	16	16
M/F	8/8	6/5	9/7	7/9
Age (Years)	65±5	65±7	66±6	67±6
Height (cm)	172±11	173±12	170±9	167±9
Weight (kg)	70±14	72±14	74±12	72±12
BMI (kg/m^2)	23.4±3.1	24.1±1	25.5±0.91	25.3±0.80
$\dot{V}O_2$ peak (ml/min/kg)	22.6±5.4	23.5±6.8	22.8±7.6	22.3±4.9
AT (%)	74±12	70±11	70±13	70±9
Daytime ABPM				
SBP (mmHg)	120±9	145±10*†	145±12*†	125±7
DBP (mmHg)	73±6	86±13*	86±8*†	77±7
MAP (mmHg)	89±7	106±11*†	105±8*†	93±6
PP (mmHg)	46±6	59±9*†	59±10*†	48±8
HR (beats/min)	73±10	69±5	69±8	68±8
Night-time ABPM				
SBP (mmHg)	108±10	129±12*†	130±12*†	112±10
DBP (mmHg)	64±6	74±10	74±5	65±5
MAP (mmHg)	80±7	93±10*†	93±9*†	82±6
PP (mmHg)	43±7	55±9*	56±11*†	47±7
HR (beats/min)	61±7	59±7	64±8	61±7
Clinic BP measurements				
SBP (mmHg)	126±8	148±17*	158±17*†	138±16
DBP (mmHg)	76±7	86±11*	89±9*†	80±9
MAP (mmHg)	93±6	106±11*	111±11*†	99±10

PP (mmHg)	50±9	62±14	68±15*	58±13
HR (beats/min)	69±11	67±11	64±12	65±11
Central BP measurements				
SBP (mmHg)	113±10	134±20*	138±17*	127±17
DBP (mmHg)	77±6	88±11*	88±11*	79±10
PP (mmHg)	36±8	46±15	50±13*	48±9*
AIx (%)	30±8	33±9	30±7	30±12
AIx75	25±9	26±9	26±8	25±10
Augmentation pressure	11±5	16±7	15±6	15±6
Pulse wave velocity				
PWV (m/s)	8.8±1.5	11.2±2*	10.6±2.5	10.6±2.3

N; number, M; male, F; female, BMI; body mass index, $\dot{V}O_2$ peak; peak volume of oxygen inspired, AT; anaerobic threshold (%), ABPM; ambulatory blood pressure monitoring, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, PP; pulse pressure, HR; heart rate, AIx; augmentation index, PWV; pulse wave velocity. * *P*=<0.05 vs. normotension; †*P*=<0.05 vs. controlled hypertension (all one-way ANOVA with Tukey post-hoc test).

Table 4-2 Assessment of multicollinearity between independent variables from a forced entry multiple linear regression test. Assessment of multicollinearity between independent variables from a forced entry multiple linear regression test assessing the influence of the change in systolic blood pressure during metaboreflex isolation (PEIO), aortic pulse pressure (aorticPP), pulse wave velocity (PWV), age and daytime ambulatory systolic blood pressure on the change in absolute systolic blood pressure during submaximal (modSBP) exercise testing (51-75% $\dot{V}O_2$ peak testing). The correlation values between independent variables were checked for large positive correlations (>0.7) before checking the tolerance statistic and variance inflation factor in Table 4.3.

Correlations

		ModSBP	PEIO	AorticPP	PWV	Age	SBP_baseline_ABPM
Pearson Correlation	ModSBP	1.000	.426	.286	.185	.046	.185
	PEIO	.426	1.000	.198	.131	.154	.367
	AorticPP	.286	.198	1.000	.519	.357	.388
	PWV	.185	.131	.519	1.000	.338	.190
	Age	.046	.154	.357	.338	1.000	.131
	SBP_baseline_ABPM	.185	.367	.388	.190	.131	1.000
Sig. (1-tailed)	ModSBP	.	.000	.018	.095	.363	.081
	PEIO	.000	.	.076	.177	.127	.003
	AorticPP	.018	.076	.	.000	.004	.002
	PWV	.095	.177	.000	.	.007	.089
	Age	.363	.127	.004	.007	.	.162
	SBP_baseline_ABPM	.081	.003	.002	.089	.162	.
N	ModSBP	59	57	54	52	59	59
	PEIO	57	57	54	52	57	57
	AorticPP	54	54	54	52	54	54
	PWV	52	52	52	52	52	52
	Age	59	57	54	52	59	59
	SBP_baseline_ABPM	59	57	54	52	59	59

Table 4-3 Assessment of multicollinearity between independent variables from a forced entry multiple linear regression test. Multiple regression assessing the influence of the change in systolic blood pressure during metaboreflex isolation (PEIO), aortic pulse pressure (aorticPP), pulse wave velocity (PWV), age and daytime ambulatory systolic blood pressure on the change in absolute systolic blood pressure during submaximal (modSBP) exercise testing (51-75% $\dot{V}O_2$ peak testing). To assess multicollinearity, the tolerance statistic should be above 0.2 and the variance should be below 10 for each independent variable. The forced entry multiple linear regression found that only the change in systolic blood pressure during metaboreflex isolation (PEIO) predicted the change in absolute systolic blood pressure during submaximal (modSBP) exercise testing (51-75% $\dot{V}O_2$ peak testing).

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	37.363	29.937		1.248	.218					
	PEIO	.616	.210	.409	2.931	.005	.426	.397	.377	.853	1.172
	AorticPP	.297	.207	.237	1.439	.157	.286	.208	.185	.614	1.629
	PWV	.390	1.058	.057	.368	.714	.185	.054	.047	.704	1.421
	Age	-.315	.394	-.113	-.800	.428	.046	-.117	-.103	.834	1.200
	SBP_baseline_ABPM	-.057	.160	-.053	-.359	.721	.185	-.053	-.046	.760	1.316

a. Dependent Variable: ModSBP

Table 4-4 Assessment of multicollinearity between independent variables from a forced entry multiple linear regression test. Multiple regression assessing the influence of the change in systolic blood pressure during metaboreflex isolation (PEIO), aortic pulse pressure (aorticPP), pulse wave velocity (PWV), age and daytime ambulatory systolic blood pressure on the change in absolute systolic blood pressure during peak exercise testing ($\dot{V}O_2$ peak testing). The correlation values between independent variables were checked for large positive correlations (>0.7) before checking the tolerance statistic and variance inflation factor in Table 4.5.

Correlations

		PeakSBP	PEIO	AorticPP	PWV	SBP_baseline_ABPM	Age
Pearson Correlation	PeakSBP	1.000	.369	.433	.318	.338	.039
	PEIO	.369	1.000	.234	.133	.353	.172
	AorticPP	.433	.234	1.000	.504	.392	.366
	PWV	.318	.133	.504	1.000	.209	.376
	SBP_baseline_ABPM	.338	.353	.392	.209	1.000	.131
	Age	.039	.172	.366	.376	.131	1.000
Sig. (1-tailed)	PeakSBP	.	.002	.001	.011	.004	.386
	PEIO	.002	.	.044	.174	.003	.101
	AorticPP	.001	.044	.	.000	.002	.003
	PWV	.011	.174	.000	.	.069	.003
	SBP_baseline_ABPM	.004	.003	.002	.069	.	.162
	Age	.386	.101	.003	.003	.162	.
N	PeakSBP	59	57	54	52	59	59
	PEIO	57	57	54	52	57	57
	AorticPP	54	54	54	52	54	54
	PWV	52	52	52	52	52	52
	SBP_baseline_ABPM	59	57	54	52	59	59
	Age	59	57	54	52	59	59

Table 4-5 Assessment of multicollinearity between independent variables from a forced entry multiple linear regression test. Multiple regression assessing the influence of the change in systolic blood pressure during metaboreflex isolation (PEIO), aortic pulse pressure (aorticPP), pulse wave velocity (PWV), age and daytime ambulatory systolic blood pressure on the change in absolute systolic blood pressure during peak exercise testing ($\dot{V}O_2$ peak testing). To assess multicollinearity, the tolerance statistic should be above 0.2 and the variance should be below 10 for each independent variable. The forced entry multiple linear regression found that the change in systolic blood pressure during metaboreflex isolation (PEIO) and aortic PP predicted the change in absolute systolic blood pressure during peak exercise testing ($\dot{V}O_2$ peak testing).

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	45.017	41.843		1.076	.288					
	PEIO	.594	.290	.269	2.044	.047	.369	.289	.248	.855	1.170
	AorticPP	.578	.285	.311	2.027	.048	.433	.286	.246	.627	1.595
	PWV	1.698	1.381	.178	1.230	.225	.318	.178	.149	.703	1.422
	SBP_baseline_ABPM	.177	.222	.110	.798	.429	.338	.117	.097	.772	1.295
	Age	-.838	.559	-.203	-1.499	.141	.039	-.216	-.182	.808	1.238

a. Dependent Variable: PeakSBP

4.7 Figures

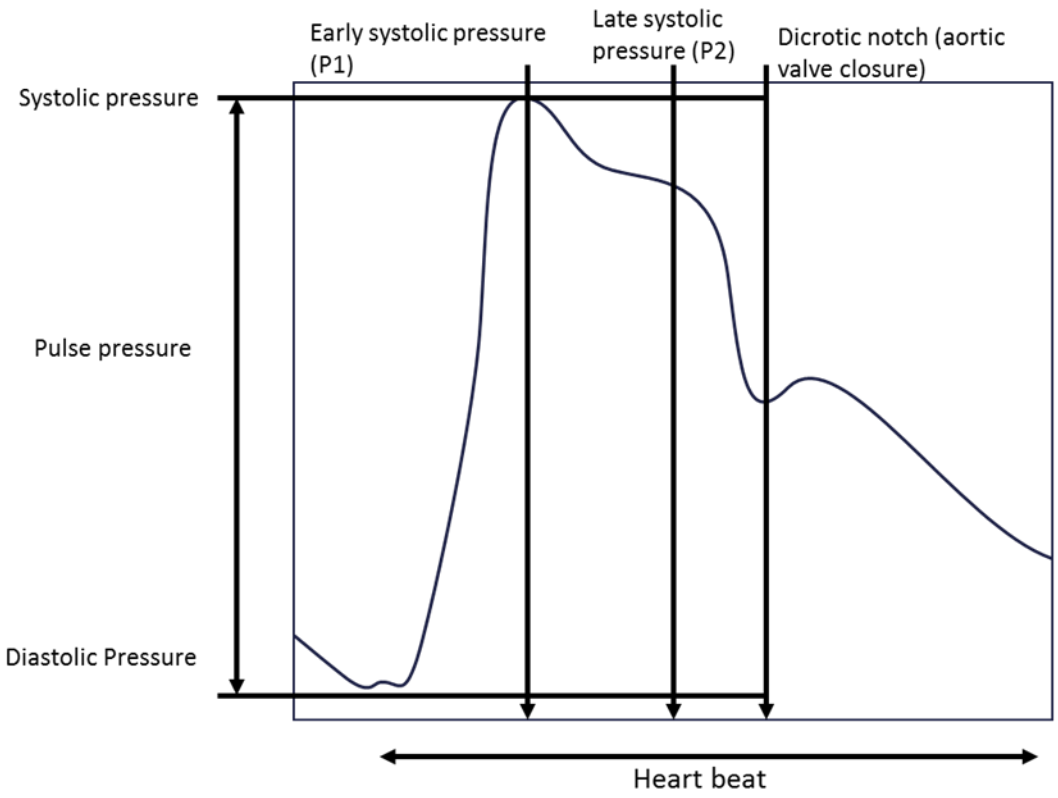


Figure 4-1 A typical central blood pressure waveform recreated from the radial pulse from an inbuilt transfer function (SphygmoCor).

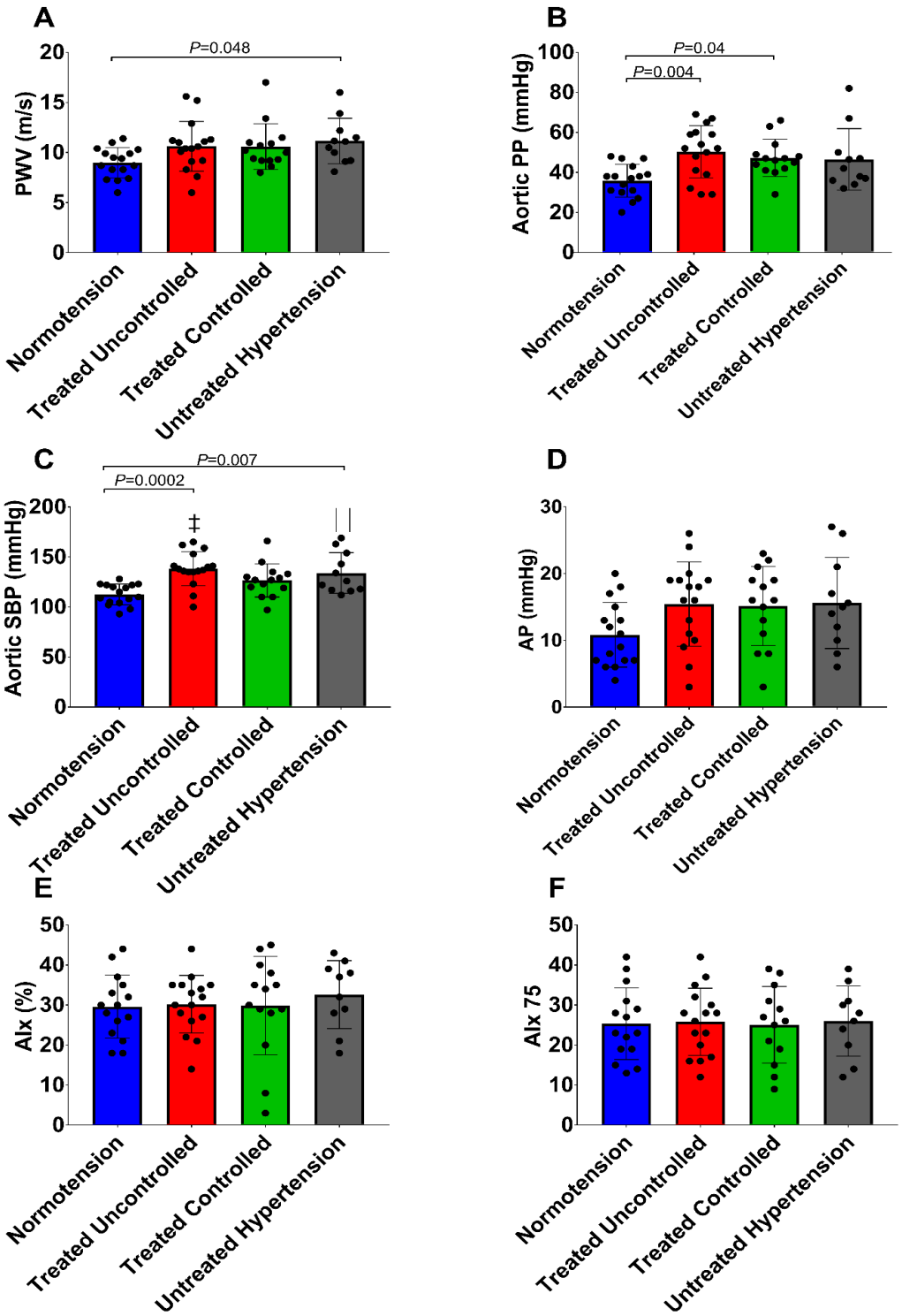


Figure 4-2 Aortic measurements from pulse wave analysis and pulse wave velocity. PWV; pulse wave velocity, Aortic PP; aortic pulse pressure, Aortic SBP; aortic systolic blood pressure, AP; augmentation pressure, AIx; augmentation index, AIx 75; augmentation index referenced to 75 beats (beats/min).

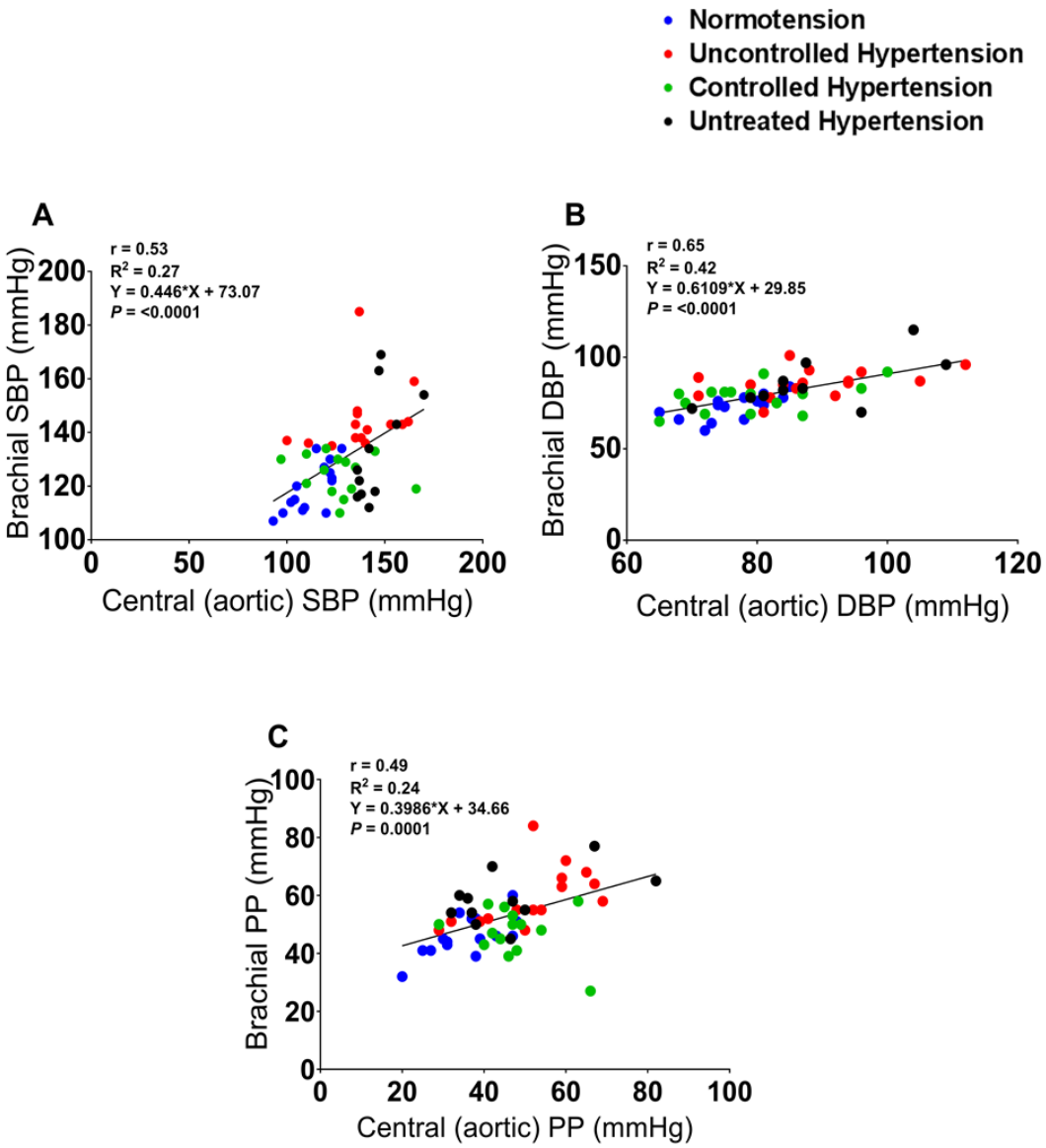


Figure 4-3 Relationship between baseline aortic haemodynamics and daytime systolic, diastolic and pulse pressure from ambulatory blood pressure monitoring. The data show that there is a positive correlation between the aortic systolic pressure (SBP) and daytime ambulatory systolic blood pressure (A). In addition, there is positive relationship between aortic DBP, aortic PP and daytime ambulatory DBP and PP respectively (B and C). Blue dots, normotensives; green dots, treated-controlled hypertensives; red dots, treated-uncontrolled hypertensives and black dots, untreated hypertensive participants.

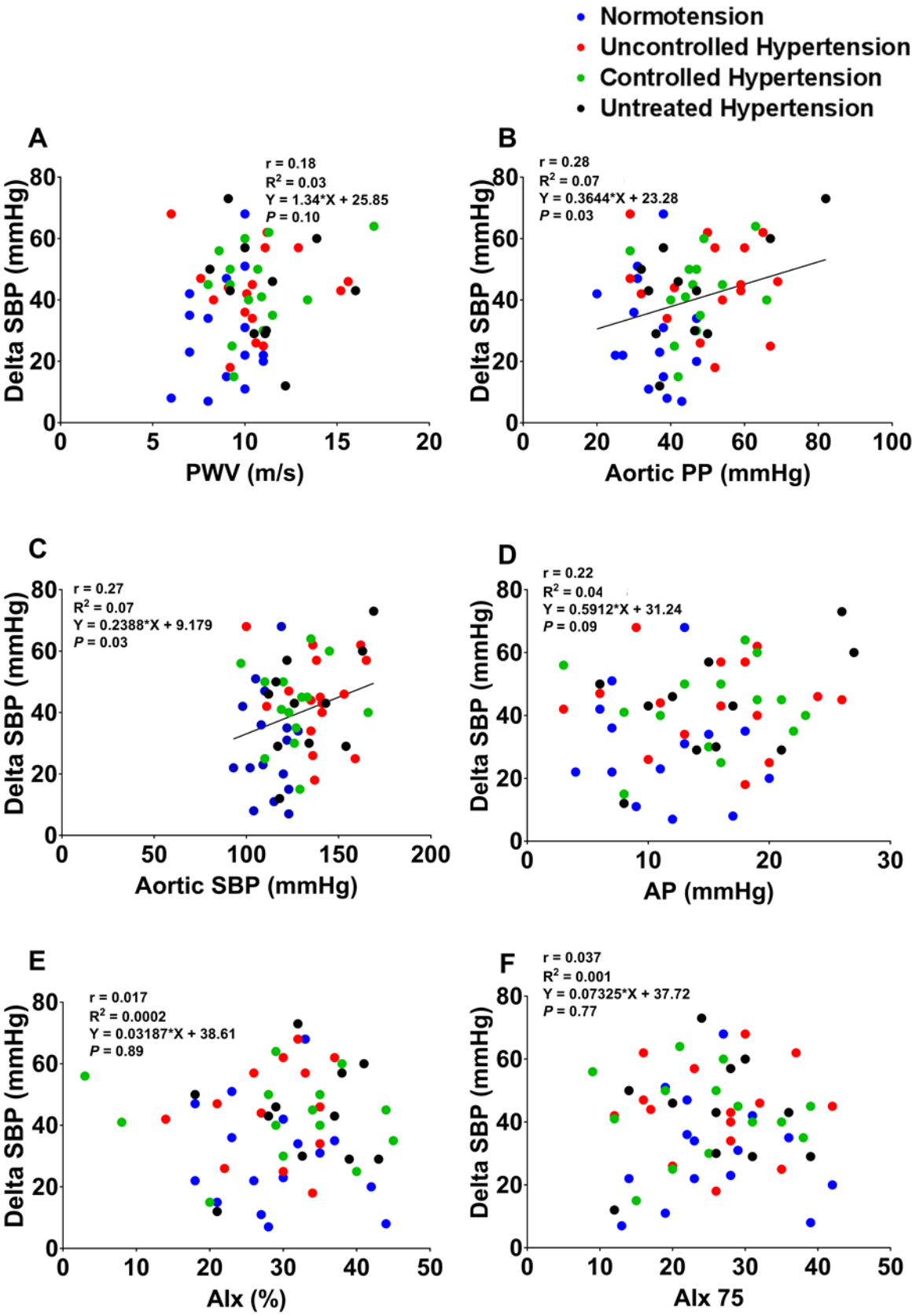

Normotension
Uncontrolled Hypertension
Controlled Hypertension
Untreated Hypertension
A
r = 0.18
R² = 0.03
Y = 1.34*X + 25.85
P = 0.10
Delta SBP (mmHg)
PWV (m/s)
B
r = 0.28
R² = 0.07
Y = 0.3644*X + 23.28
P = 0.03
Delta SBP (mmHg)
Aortic PP (mmHg)
C
r = 0.27
R² = 0.07
Y = 0.2388*X + 9.179
P = 0.03
Delta SBP (mmHg)
Aortic SBP (mmHg)
D
r = 0.22
R² = 0.04
Y = 0.5912*X + 31.24
P = 0.09
Delta SBP (mmHg)
AP (mmHg)
E
r = 0.017
R² = 0.0002
Y = 0.03187*X + 38.61
P = 0.89
Delta SBP (mmHg)
AIx (%)
F
r = 0.037
R² = 0.001
Y = 0.07325*X + 37.72
P = 0.77
Delta SBP (mmHg)
AIx 75

Figure 4-4 Relationship between baseline central haemodynamics and the change in peripheral SBP during submaximal dynamic exercise (51-75% $\dot{V}$O2 peak testing).

The data show that there is a positive correlation between the aortic pulse pressure (PP) and aortic systolic blood pressure (SBP) and the change in peripheral SBP during submaximal dynamic exercise (51-75% $\dot{V}O_2$ peak testing). Blue dots, normotensives; green dots, treated-controlled hypertensives; red dots, treated-uncontrolled hypertensives and black dots, untreated hypertensives.

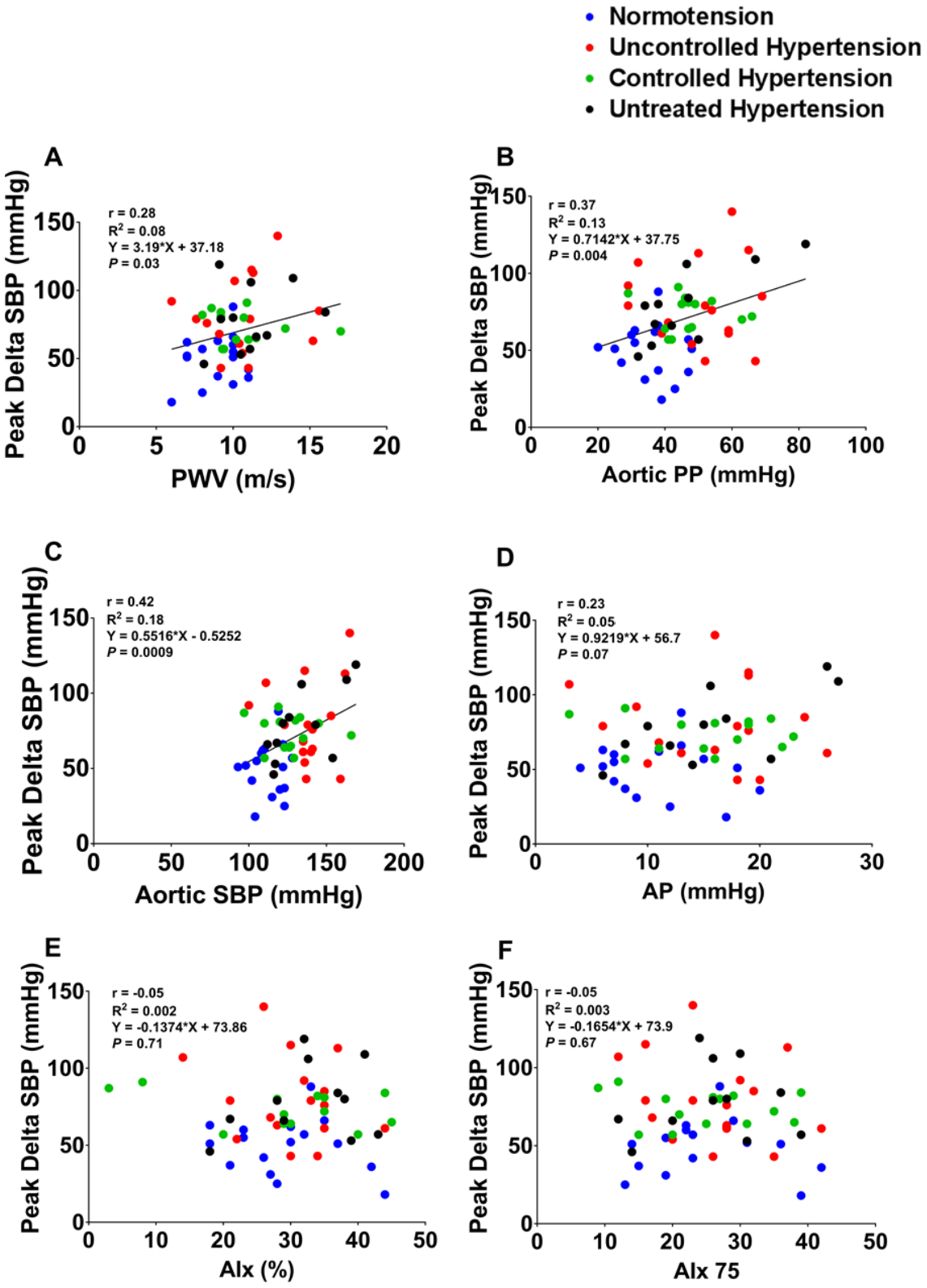


Figure 4-5 Relationship between baseline central haemodynamics and the change in peripheral systolic blood pressure (SBP) at peak dynamic $\dot{V}O_2$ peak testing).

The data show that there is a positive correlation between pulse wave velocity, aortic pulse pressure (PP) and aortic systolic blood pressure (SBP) and the change in peripheral SBP during peak dynamic exercise ($\dot{V}O_2$ peak testing). Blue dots, normotensives; green dots, treated-controlled hypertensives; red dots, treated-uncontrolled hypertensives and black dots, untreated hypertensive participants.

Chapter 5 Can dietary nitrates prevent excessive rises in blood pressure in people with treated-controlled hypertension?

5.1 Introduction

The study in Chapter 3 demonstrated that patients with treatment controlled hypertension had an exaggerated rise in systolic blood pressure (SBP) during incremental exercise testing which was similar to patients with untreated hypertension (Chant et al., 2018). Importantly, both groups had an elevated rise in SBP compared to normotensive individuals (Chant et al., 2018). This was, in part, due to increased metaboreflex activation during exercise in the patients with hypertension (Chant et al., 2018). This finding is concerning because an exaggerated blood pressure (BP) response to exercise is independent risk for adverse cardiovascular (CV) events (Kjeldsen et al., 2001, Kjeldsen et al., 1997, Kurl et al., 2001, Laukkanen et al., 2006, Ren et al., 1985, Mizuno et al., 2016a, Mundal et al., 1996, Filipovsky et al., 1992). Therefore, first line treatment for hypertension clearly does not target the metaboreflex and alternative therapies need to be developed to make exercise safer in hypertension.

The increased sympathetic nerve activity (SNA) that occurs during exercise is directed to all vascular beds (active and inactive). Vasoconstriction in non-metabolically active areas helps to maintain perfusion pressure into the active skeletal muscle. In the active skeletal muscle, the increased SNA is offset by vasoactive metabolites in a process known as functional sympatholysis (Thomas et al., 1994), which helps to ensure adequate perfusion pressure of the active muscle. Functional sympatholysis is impaired in patients with untreated

hypertension (Vongpatanasin et al., 2011, Price et al., 2013). Impaired functional sympatholysis is partially mediated by reduced nitric oxide (NO) bioavailability in animal models of hypertension (angiotensin II infused rats) (Zhao et al., 2006) and spontaneously hypertensive rats (SHRs) (Mizuno et al., 2014a). In hypertensive humans nebivolol, a non-specific β adrenoreceptor antagonist with NO potentiating abilities (Münzel and Gori, 2009) improves functional sympatholysis during dynamic handgrip exercise with lower body negative pressure (-20 mmHg) in untreated hypertensives (Price et al., 2013). Research indicates that reductions in blood flow to the active skeletal muscle during exercise have been shown to increase metaboreflex hyperreflexia (Kaufman et al., 1984, Xing et al., 2013). For example, ligation of the femoral artery in rats leads to an increased expression of acid sensing ion channel 3 (ASIC3) (Liu et al., 2010), purinergic receptor 3 subtype (P_2X_3) (Xing et al., 2013) and bradykinin B2 (Lu et al., 2013) on the group IV (metaboreflex) afferents, which augments metaboreflex sensitivity. This suggests that improving blood flow responses to exercise, by enhancing NO bioavailability in hypertensive individuals could decrease the activation of the metaboreflex during exercise.

Increasing NO bioavailability is possible through dietary nitrates (NO_3^-) (e.g. beetroot, spinach, rocket) (Demoncheaux et al., 2002, Webb et al., 2008b). This is known as the NO_3^--nitrite (NO_2^-)-NO pathway. NO_3^- is reduced by symbiotic bacteria reduce NO_2^- on the posterior portion of the tongue (Duncan et al., 1995). The reduction of NO_2^- to NO is endothelial independent and is favoured under conditions of hypoxia, acidity and reduced oxygen (O_2) tension (Shiva, 2013); ideal conditions for the skeletal muscle during exercise (Piknova et al., 2016).

Consumption of dietary NO_3^- has been shown to lower BP in placebo controlled, double-blind, randomised and crossover design studies in patients with hypertension (Kapil et al., 2015, Ghosh et al., 2013). Importantly, in pre-hypertensive males, dietary NO_3^- consumption for 15 days increased total vascular conductance and decreased the BP response to submaximal cycle ergometer testing (Choi et al., 2016). In addition, dietary NO_3^- have also been shown to lower SNA during isometric handgrip exercise (Notay et al., 2017) and metaboreflex activity (post-exercise ischemia (PEI)) in healthy individuals (Schneider et al., 2018). Currently, it is not understood whether dietary NO_3^- can lower the metaboreflex hyperreflexia and exercise BP in treated controlled hypertensive patients. By increasing NO bioavailability and improving functional sympatholysis, dietary NO_3^- could be expected to decrease metaboreflex hyperreflexia and the BP response to peak exercise testing ($\dot{V}O_2$ peak testing) in treated controlled hypertension.

Therefore, the main aim of this study was to assess whether 4 weeks of dietary NO_3^- can improve the SBP at peak exercise ($\dot{V}O_2$ peak testing) in treated controlled hypertension compared to a placebo. The secondary aim was to assess whether 4 weeks of dietary NO_3^- can improve metaboreflex hyperreflexia during PEI compared to a placebo. The tertiary aim was to evaluate whether dietary NO_3^- for 4 weeks will change the MSNA (bursts/min) and MSNA (bursts/100Hb) during PEI compared to a placebo. The quaternary aim was to measure whether the change in MSNA (bursts/min) and MSNA (bursts/100Hb) during quiet resting was changed following dietary NO_3^-compared to a placebo. It was hypothesised that 4 weeks of dietary NO_3^- will cause a change in SBP at

peak exercise ($\dot{V}O_2$ peak testing) when compared to a placebo (primary hypothesis). In addition, 4 weeks of dietary NO_3^- will cause a change in SBP during isolation of the metaboreflex (PEI2). The tertiary hypothesis was that 4 weeks of dietary NO_3^- will cause a change in MSNA (bursts/min) and MSNA (bursts/100Hb) during metaboreflex isolation (PEI2). The quaternary hypothesis was that 4 weeks of dietary NO_3^- will cause a change in resting MSNA (bursts/min) and MSNA (bursts/100Hb) at baseline following 4 weeks of dietary NO_3^- compared to placebo.

5.2 Methods

5.2.1 Participants

5.2.1.1 Beetroot juice study

36 treated controlled hypertensive participants were screened for this study. 21 participants were recruited and a further 15 were excluded due to screening failure (Figure 5.1, page 309). 12 participants were randomly allocated to the dietary NO_3^- group and 9 participants were allocated to the placebo group. All participants were matched for age, body mass index (BMI) and for CV fitness ($\dot{V}O_2$ peak) (Table 5.1, page 301). Overall, 11 females (52%) were recruited to this study and all of which were postmenopausal (100%). This study was designed as a pilot study for a future project grant application to the British Heart Foundation (BHF). Ethical approval was granted by the Northern-Ireland proportionate review NHS REC (17/NI/0097) and received local R&D approval. Volunteers were recruited from our groups' specialist hypertension clinic at University Hospitals Bristol Trust and Foundation, Bristol. The remaining participants were recruited from the surrounding area. The

study was conducted at the Clinical Research and Imaging Centre (CRiC), Bristol. All participants attended the CRiC-Bristol at a similar time of day and all lab conditions were controlled to a set temperature (22°C). Participants were asked to refrain from alcohol and caffeine for 12 hours before the study visits. In addition, participants were asked to abstain from high-intensity exercise for at least 24-hours prior to participation. The participants were asked to take their anti-hypertensive medication as normal.

Inclusion and exclusion criteria: In accordance with the National Institute for Health and Care Excellence (NICE) guidelines (NICE, 2011), treated-controlled hypertension was defined as taking one or more anti-hypertensive medications and adequate BP control on daytime ambulatory blood pressure monitoring (ABPM) (<135/85 mmHg). Similar to the study in Chapter 3, exclusion criteria included; diabetes mellitus (urine dipstick test and self-reported), BMI (>30 kg/m^2), pregnancy, major illness (such as cancer), overt respiratory-CV disease (other than hypertension), and febrile illness with 2 weeks of the study. Participants were also asked to avoid the use of painkillers such as aspirin, paracetamol or anti-inflammatory drugs (e.g., ibuprofen) for 24 hours prior to the study visits. Participants were asked to refrain from these medications due to their known inhibitory effects on exercise BP (Drew et al., 2013, Cui et al., 2007, Cui et al., 2008b). For this study additional exclusion criteria included: i) current use of antibiotics or antibiotic use with the last 3 months, ii) current use of antibacterial mouthwash, iii) use of proton pump inhibitors (including esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole) and xanthine oxidase inhibitors, iv) currently consuming dietary NO_3^-based products

(e.g., beetroot juice). Participants currently using antibiotics, antibacterial mouthwash and proton pump inhibitors are excluded from this study as these are known to alter the bacteria that convert NO_3^- to NO_2^-, which is vital for inorganic NO_3^- to have its therapeutic benefit (Woessner et al., 2016, Kapil et al., 2013).

5.2.1.2 Study design and randomisation

This was a single-centre, double-blinded, randomised placebo-controlled trial with a parallel group design. The researcher remained blinded until the data analysis was complete. Volunteers were randomised to receive the active and placebo beetroot juice in a 1:1 ratio with concealment using a binary random number sequence by a study co-coordinator who was not involved in the data collection, analysis or interpretation of data.

5.2.2 Dietary Nitrates

Participants were randomly allocated to take one x 70 mL/day of active beetroot juice (Beet It Sport; James White Drinks Ltd) containing ~6 mmol of NO_3- or one x 70 mL/day of a NO_3^- depleted placebo beetroot juice (Beet It Sport; James White Drinks Ltd) with breakfast for 4-weeks in total. This was a parallel design study; participants took the active **or** the placebo NO_3^- depleted beetroot juice. Importantly, both the active and placebo beetroot juice can produce discolouration of the urine and stool. The NO_3^--depleted placebo control is identical in appearance, taste and causes similar side effects which ensured that participants remain blinded to the treatment group (active placebo). Following the 4-week intervention, participants returned for post-assessment one which involved fitting a 24-hour ABPM again. The participant then returned for a final visit (post-

assessment two) which was a repeat of pre-assessment two. Participants were instructed to take their final supplement on the morning of post-assessment two. A flow diagram of pre- and post-assessment two can be found in Figure 5.3 (page 311).

A 6 mmol/day dose of dietary NO_3- was chosen due to a recent study which found that the O_2 cost ($\dot{V}O_2$) of submaximal exercise was reduced in healthy individuals when consuming 6 mmol of NO_3- per day for 4-weeks but not 3 mmol of NO_3- per day (Wylie et al., 2016). Similarly, Kapil et al. (2015) found that 24-hour ambulatory BP was reduced in treated and drug-naïve hypertensive patients after consumption of 6 mmol/day of dietary NO_3^- for 4-weeks compared to placebo. A recent meta-analysis suggested that a longer duration of beetroot juice (>14 days) had a more profound effect on lowering resting BP compared to <14 days (Bahadoran et al., 2017). Therefore, based on this previous evidence we chose one x 70 mL of Beet It Sport for 4-weeks which contains ~6 mmol of NO_3- per day.

5.2.3 Screening procedures

Participants attended four visits for this study. Pre-assessment one and two and post-assessment one and two. The screening procedures were carried out at pre-assessment one. A summary of each visit can be found in Table 5.2 (page 303). A flow diagram for pre-assessment one can be found in Figure 5.2 (page 310). Participants attended a screening visit where resting clinic BP was measured using an automated cuff (Omron, The Netherlands). In accordance with the European Society of Hypertension (O'Brien et al., 2000, O'Brien et al., 2001,

O'Brien et al., 2013), the first BP measurement was ignored which was followed by a BP reading on both the left and right arm and a final reading was then taken on the arm where BP was highest. An average of the final two readings was taken as clinic BP. A research nurse then fitted participants with a 12-lead electrocardiogram (ECG) to rule out any CV abnormalities. The ECG was checked by a cardiologist. Participants were fitted with a 24-hour ABPM to ensure that participants were classified as treated controlled hypertension (Spacelabs, OSI Systems Company, USA). The 24-hour ABPM assessed BP every 30 minutes during the daytime and once per hour throughout sleeping hours (O'Brien et al., 2000, O'Brien et al., 2013, O'Brien et al., 2001). In addition, participants completed a 24-hour BP diary. Participants were asked to avoid any exercise during this 24-hour period to avoid post-exercise hypotension (Brito et al., 2014).

5.2.4 Study procedures

An outline of pre- and post-assessment two can be found in Figure 5.3 (page 311). Once the daytime ABPM was confirmed as treated controlled hypertension (SBP<135/85 mmHg) participants were asked to return for a follow up visit (pre-assessment two) at least 48 hours after pre-assessment one. Participants were asked to follow a low- NO_3^- diet for 24 hours prior to arrival at the laboratory for pre-assessment two for the assessment of baseline plasma NO_3^- and NO_2^- levels (see section 5.2.4.1, Biological sample collection, page 264). Upon arrival, participants were asked to be supine on the bed prior to a venous sample being taken for the assessment of baseline plasma NO_3^- and NO_2^- levels (see section

5.2.4.1, Biological sample collection, page 264; Figure 5.3, page 311). Following this, clinic BP was assessed using the same protocol from pre-assessment one. The maximal voluntary contraction (MVC) was then assessed using a handgrip dynamometer on the dominant hand. To assess the MVC, participants performed a maximal contraction of the handgrip dynamometer three times with at least 30 seconds between each attempt. The highest value of the three attempts was regarded as the MVC (Delaney et al., 2010). The MVC was assessed to calculate 40% MVC for the isometric handgrip exercise. An outline of pre-assessment two can be found in Figure 5.3 (page 311). Microneurography was then attained in the participants prior to isometric handgrip exercise and metaboreflex testing. After a 15-minute rest, a $\dot{V}O_2$ peak test was then completed on a cycle ergometer (see section 5.2.4.4. $\dot{V}O_2$ peak testing, page 267).

5.2.4.1 Biological sample collection:

To confirm whether participants had been taking the NO_3^--rich beetroot juice or the NO_3^--depleted beetroot juice supplement, NO_3^-/NO_2^- concentrations from venous plasma were tested (Kapil et al., 2015). Baseline levels of NO_3^- and NO_2^- were tested following a 24-hour low NO_3^- diet to ensure similar baseline levels between the NO_3^--rich and placebo group. During this time participants were asked to keep a food diary. Participants then returned after 4-weeks for testing of venous plasma levels of NO_3^- and NO_2^-. Participants were asked again to follow a low dietary NO_3^- diet for 24-hours prior to this visit (except for the NO_3^--rich beetroot juice or NO_3^--depleted placebo). Dietary NO_3^- has a half-life of ~6-hours (Webb et al., 2008b). NO_2^- peaks at around 3-hours following ingestion (Webb et al., 2008b). However,

the plasma NO_2^- levels will remain elevated for around 6-hours in healthy (Webb et al., 2008b) and in hypertensive (Ghosh et al., 2013) individuals. Therefore, in the follow up visits following 4-weeks of supplementation we asked both the dietary-NO_3^- rich beetroot juice and NO_3^--depleted beetroot juice group to take their final supplement 1.5 hours prior to attending the laboratory.

Blood sample collection and storage: Participants blood was collected using standard venepuncture from the median cubital vein into two paediatric 2mL lithium heparin vacutainer tubes and immediately centrifuged (1500g, 4°C, 10 minutes) to separate plasma from red blood cells. Using filtration, the samples were deproteinated using 3kDa filters (Vivaspin 500, Sartorius Biotech, Aubagne, France) (14000g, 4°C, 60 minutes). The resulting filtrate was then stored at -80°C until analysis at a later date. This procedure was followed in accordance with Dr. Kapil's (Kapil et al., 2010, Kapil et al., 2013, Kapil et al., 2015) laboratory.

5.2.4.1.1 Nitrate/nitrite concentration:

Determination of NOx: The total concentration of NO_3^- and NO_2^- (NOx) was determined using ozone based chemiluminescence (Ignarro et al., 1993, Bush et al., 1992) (Figure 5.4, page 312). Total NOx (NO_3^- and NO_2^-) concentration was determined by adding biological samples to 0.1 mol/L vanadium (III) chloride in 1 mol/L hydrochloric acid refluxing at 95°C under nitrogen (inert gas) (Kapil et al., 2015). Both NO_3- and NO_2- are reduced to NO in acidic vanadium (III) chloride (Ignarro et al., 1993). NO present or produced is then carried by an inert gas (nitrogen) to the reaction cell. NO that is produced reacts with ozone (O_3) in the

reaction cell to form nitrogen dioxide (NO_2) in an excited state (NO_2*) (Coneski and Schoenfisch, 2012). The subsequent decaying and relaxation of NO_2* to NO_2 releases a photon (h*Y)* that is detected by a photomultiplier tube (Coneski and Schoenfisch, 2012). This can be summarised by the following equations:

$NO + O_3 \longrightarrow NO_2^* + O_2$ [equation 5.1]

$NO_2^* \longrightarrow NO_2 + hY$ [equation 5.2]

* indicates an excited state of the NO_2-.

The signal detected by the chemiluminescence procedure is proportional to the level of NO and therefore NOx (Coneski and Schoenfisch, 2012). Samples measured were then compared to a standard calibration curve generated from a commercially available premixed known concentration of NO gas.

Determination of nitrate and nitrite concentration: NO_2- concentrations can be directly determined by refluxing 1% potassium iodide in glacial acetic acid (Ignarro et al., 1993). Under these conditions NO_2- is reduced to NO, however, NO_3- concentrations cannot be detected as NO_3- cannot be directly reduced to NO (Ignarro et al., 1993). Therefore, to calculate NO_3- concentrations:

$NO_3- = NO_x – NO_2-$ [equation 5.3]

Levels of NO_3- and NO_2- (µmol/L) from plasma will be reported as absolute values at both baseline and following 4-weeks of supplementation.

This analysis took place in Dr Vikas Kapils laboratory at Queen Mary University of London (an expert in NO_3^- metabolism) where NO_3^- and NO_2^- levels are regularly measured (Kapil et al., 2013, Kapil et al., 2015, Kapil et al., 2010).

5.2.4.2 Microneurography

Multiunit efferent muscle sympathetic nerve activity (MSNA) was assessed using a tungsten microelectrode positioned in the peroneal nerve at the fibular head. For expanded information regarding the microneurography procedure see Chapter 2 (section 2.7, page 117).

5.2.4.3 Isometric handgrip and Metaboreflex testing

Following a baseline period of 10 minutes participants were asked to perform isometric handgrip at 40% MVC for 1 minute. An occlusion cuff was then pumped up to supra-systolic pressures (240) mmHg and this remained inflated following isometric handgrip exercise for 2 minutes and was regarded as post-exercise ischemia (PEI) (Delaney et al., 2010, Sausen et al., 2009, Greaney et al., 2014, Alam and Smirk, 1937). For a more detailed information regarding isometric handgrip exercise and metaboreflex testing see Chapter 2 (section 2.6, page 108).

5.2.4.4 $\dot{V}O_2$ peak testing

Participants were asked to rest for 15 minutes prior to the $\dot{V}O_2$ peak test. BP was assessed every 1.5 minute during $\dot{V}O_2$ peak testing on the left arm of the participant using an automated sphygmomanometer (Love Medical, Manchester, UK). Heart

rate (HR) was assessed using a 12-lead ECG (Love Medical, Manchester, UK). Breathing frequency, tidal volume, minute ventilation (breathing frequency * tidal volume), $\dot{V}O_2$ and volume of carbon dioxide expired ($\dot{V}ECO_2$) were assessed using an Ergoflow flow sensor for spirometry (Ergostik CPET system, Love Medical, Manchester, UK). The same protocol was used to assess $\dot{V}O_2$ peak that was used in Chapter 3 (Chapter 2; section 2.3.1, page 94 and Chapter 3; section 3.2.1, page 151). See Chapter 3 (section 3.2.1, page 151) for the criteria used to define $\dot{V}O_2$ peak.

5.2.4.5 Physiological Monitoring During $\dot{V}O_2$ Peak and Metaboreflex Testing

5.2.4.5.1 $\dot{V}O_2$ peak test

The methods used for physiological assessment during the $\dot{V}O_2$ peak test can be found in chapter 3 (section 3.2.3.1, page 154).

5.2.4.5.2 Handgrip and Metaboreflex isolation

See Chapter 3 for methods used for physiological assessment during handgrip, and metaboreflex isolation (PEI) (section 3.2.4.1, page 155). Additionally, for this study the change in MSNA (bursts/min, bursts/100Hb and total MSNA/min and total MSNA/Hb) were compared to a baseline period prior to isometric handgrip exercise. The change in BP was assessed on a beat-to-beat basis using finger plethysmography (Finometer; Finapres Medical Systems, The Netherlands, Chapter 2, section 2.6, page 108). The change in HR was measured using a 3-lead ECG. Finally, the change in stroke volume (SV), cardiac output (CO) and total

peripheral resistance (TPR) were assessed using modelflow from the Finapres (Finometer; Finapres Medical Systems, The Netherlands, Chapter 2, section 2.6.2, page 111).

5.2.5 Power calculations

To test the hypothesis, 20 participants with treated-controlled hypertension in each group (n=20 for intervention and n=20 for placebo) will provide the power of >80% to find a statistical difference ($P \leq 0.05$) in peak SBP during maximal exercise testing after 4-weeks intervention period of NO_3^--rich beetroot juice compared to a placebo control (NO_3^- depleted beetroot juice). This is based on previous results (Kapil et al., 2015) indicating that clinic SBP was reduced by 8.7±8.2 mmHg following NO_3^- rich beetroot juice (n=32) compared to a 1±8.4 mmHg reduction following placebo control (n=32) (NO_3^- depleted beetroot juice) in individuals with hypertension. From these previous results a large effect size was calculated (d=0.93). See results section for information regarding why recruitment was stopped prematurely.

5.2.6 Outcomes

The primary end point of this study was the difference in SBP at peak exercise from pre to post intervention ($\dot{V}O_2$ peak test). Secondary end points were the change in SBP and MSNA (bursts/min) and MSNA total activity (arbitrary units (au)) from pre to post intervention during metaboreflex isolation (PEI). The change in diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP), heart rate (HR), tidal volume, breathing frequency, minute

ventilation and the respiratory efficiency slopes were also considered during $\dot{V}O_2$ peak testing. Similarly, DBP, PP, MAP, HR, heart rate variability, cardiac and sympathetic baroreflex sensitivity and sympathetic transduction were considered during metaboreflex isolation (PEI).

5.2.7 Data analysis

5.2.7.1 $\dot{V}O_2$ peak test

The methods used to analyse the $\dot{V}O_2$ peak test are described in Chapter 3 (section 3.2.7.1, page 157).

5.2.7.2 Isometric handgrip, metaboreflex testing and recovery

The absolute change in SBP, DBP, mean arterial pressure (MAP), pulse pressure (PP), HR, SV, CO, TPR and MSNA (bursts/min, bursts/100hb, MSNA area and total MSNA) were averaged over 1-minute periods. The change in SBP, DBP, MAP, PP, HR, SV, CO, TPR and MSNA (bursts/min, bursts/100hb, MSNA area and total MSNA) were assessed by comparing to a baseline period prior to the onset of isometric handgrip exercise. PEI was split into 2x1 minute periods which accounted for the initial drop in BP following the withdrawal of isometric handgrip exercise expected in PEI1 (Delaney et al., 2010). The second minute of post-exercise ischemia (PEI2) was used for analysis of the metaboreflex. When isometric HG stops, there is an initial drop in BP that may activate the baroreflex and therefore isolation of the metaboreflex cannot be guaranteed (Delaney et al., 2010). However, BP rises again and remains stable during the second minute of

PEI2. Recovery was split into 5x1 minute periods for the assessment of recovery SBP, DBP, MAP, PP, HR, SV, CO, TPR and MSNA (bursts/min, bursts/100hb, MSNA area and total MSNA).

5.2.7.3 Identifying and quantifying MSNA (bursts/min, bursts/100Hb, MSNA area and total MSNA)

MSNA burst identification was completed using a script written in a data analysis program (Spike 2, Cambridge Electronic Designs). More specific details regarding MSNA burst identification can be found in Chapter 2 (section 2.7, page 117).

5.2.7.4 Additional physiological measurements

5.2.7.4.1 Sympathetic vascular transduction

Noradrenaline released from the sympathetic nerves leads to vasoconstriction of resistance vessels and increased arterial resistance. This transfer of sympathetic activity into vascular tone is known as sympathetic vascular transduction (Briant et al., 2016). To quantify this the relationship between MSNA and DBP was assessed. DBP is used for this analysis because DBP has a stronger correlation with MSNA than SBP (Sundlöf and Wallin, 1978). This analysis was performed using an automated script in MATLAB (The MathWorks, Natick, MA, USA). First the neurogram was normalised. Burst area was calculated by firstly assigning the largest burst in the signal as 100 arbitrary units (AU) and a period of no bursts was marked as 0 AU (Hart et al., 2017). The start and end of each multiunit

MSNA burst were then marked and the integral was then assessed between 'start' and 'end' (Hart et al., 2017). More specifically, for each DBP the MSNA burst area was calculated during a fixed cardiac interval. The slope of MSNA burst area (arbitrary units; au) against DBP (mmHg) was then calculated using weighted linear regression (Briant et al., 2016). The slope of the line was taken as the sympathetic vascular transduction (Briant et al., 2016). To assess the optimal cardiac cycle to use for this analysis, the sympathetic vascular transduction analysis was performed for each defined cardiac cycle (3–1, 4–2, 5–3, 6–4, 7–5, 8–6, 9–7 and 10–8) during both baseline and recovery (Figure 5.5, page 313). Similar to previous research (Briant et al., 2016) a cardiac cycle of 8-6 was used for analysis of the sympathetic vascular transduction (Figure 5.5, page 313). A previous study using this technique found that sympathetic vascular transduction increased from young women to older women whereas sympathetic vascular transduction decreased in men with age (Briant et al., 2016). As NO may influence sympathetic vascular transduction, the pre-dietary NO_3^- vs post dietary NO_3^- data will be compared. Sympathetic vascular transduction was measured during baseline (10 minutes) and recovery from PEI (5 minutes).

5.2.7.4.2 Spontaneous sympathetic baroreflex sensitivity

The sensitivity of the sympathetic baroreflex can be assessed by quantifying spontaneous changes in DBP and whether this triggers changes in MSNA (Hart et al., 2010). Changes in DBP are used because increases in MSNA are initiated when baroreflex inhibition wears off during diastole (Wallin and Nerhed, 1982). More specifically, the DBP was first calculated for each cardiac cycle and put into

1 mmHg bins. Next, each DBP that was associated with an MSNA burst was calculated. If no MSNA burst occurred during the cardiac cycle, then the burst was assigned a value of 0. A weighted linear regression of the % of 1 mmHg DBP that were associated with a burst and the total number of cardiac cycles was then performed. The slope of the weighted linear regression was then taken as the sensitivity of the sympathetic baroreflex (Hart et al., 2010). The sensitivity of the baroreflex was calculated during baseline (10 minutes) and during recovery (5 minutes) from metaboreflex testing. An example can be found in Figure 5.6 (page 314).

5.2.7.4.3 Heart Rate Variability

The methods used to analyse heart rate variability are described in Chapter 3 (section 3.2.8.1, page 159).

5.2.7.4.4 Spontaneous Cardiac Baroreflex Sensitivity

The methods used to analyse spontaneous cardiac baroreflex sensitivity are described in Chapter 3 (section 3.2.8.2, page 160).

5.2.8 Statistical Analysis

The difference in baseline parameters (daytime BP, night time BP and clinic BP) from pre- to post-treatment was compared between placebo and dietary NO_3^- groups using an unpaired students t-test.

Primary outcome: For this study the main outcome was the difference in the absolute SBP at peak exercise ($\dot{V}O_2$ peak testing) from pre- to post-treatment. This was compared between placebo and dietary NO_3^- groups using an unpaired students t-test. Magnitude based inferences were carried out to determine the effect of the active treatment on the primary outcome. A qualitative inference of beneficial, trivial or harmful effect of active treatment was given as follows 0-5% (very unlikely), 5-25% (unlikely), 25-75% (possibly), 75-95% (likely), 95-99.5% (very likely) and > 99.5% (most likely) (Batterham and Hopkins, 2006).

Secondary outcomes: The difference in the absolute DBP, PP, MAP, HR, tidal volume, breathing frequency and minute ventilation at peak exercise from pre- to post-treatment were also analysed using an unpaired t-test.

The absolute change and % change in SBP, DBP, PP, MAP, HR, tidal volume, breathing frequency and minute ventilation from baseline during the different exercise intensities (% $\dot{V}O_2$ peak testing) were tested using a 2-way mixed model ANOVA (within-subject (time (exercise intensity)) and between-subject (treatment)). A *post-hoc* Tukey test was used if a significant interaction was found.

The difference in the absolute SBP during metaboreflex isolation (PEI2) from pre- to post-treatment was compared using an unpaired students t-test. The difference in the absolute DBP, PP, MAP, HR, SV, CO, TPR and MSNA (bursts/min, bursts/100hb, MSNA area and total MSNA) during PEI2 from pre- to post-treatment were also analysed using an unpaired t-test.

The group averages for the absolute change in SBP, DBP, MAP, PP, HR, SV, CO, TPR and MSNA (bursts/min, bursts/100hb, MSNA area and total MSNA) from baseline during isometric handgrip exercise and the two 1-minute periods of PEI were compared using a mixed model 2-way ANOVA (within-subject (time (isometric handgrip or PEI) and between-subject (treatment)). A post-hoc Tukey test was used if a significant interaction was found.

All data from the $\dot{V}O_2$ peak test, isometric handgrip and PEI are reported as mean ± standard deviation (SD). The Cohens effect sizes (*d*) and eta squared (η^2) were calculated to assess the magnitude of the differences. The magnitude of the *d* and η^2 were based on the following criteria: <0.2 (trivial), 0.2-0.6 (small effect), 0.6-1.2 (moderate effect), 1.2-2 (large effects) and >2 a very large effect. Data were tested for normal distribution using a D'Agostino-Pearson omnibus K2 normality test. The α level was set at 0.05.

5.3 Results

This study was stopped before the correct number of participants were collected to fulfil the sample size due to time constraints in the completion of this thesis. 21 participants with treated controlled hypertension have completed this study. 12 in the active dietary NO_3^- group and 9 in the placebo group. Recruitment of participants with treated-controlled hypertension was much more difficult than previously expected (Figure 5.1, page 309).

5.3.1 *Pre/post-treatment participant demographics*

Pre-treatment age was similar between the placebo and dietary NO_3^- group (*t*(19) = 0.19; *P*=0.85; Table 5.1, page 301). Pre and post treatment BMI scores remained similar following dietary NO_3^- (26.2±3.1 (pre) vs 25.9±2.8 (post) kg/m^2) and after placebo (26.7±3.2 (pre) vs 26.5 (post) kg/m^2) ($F(1,19)=3.34$; $P=0.83$; $\eta^2 = 0.150$).

At pre-treatment daytime ambulatory SBP was similar between the placebo (124±8 mmHg) and dietary NO_3^- group (125±8 mmHg) prior to intervention (*t*(19) = 0.15; *P*=0.87; *d*=0.125 Table 5.1, page 301). Daytime DBP was also similar between placebo and dietary NO_3^- (79±11 vs 80±7 mmHg, *t*(19) = 0.03; *P*=0.97; *d*=0.11). Similar results were found for MAP (*t*(19) = 0.13; *P*=0.91; *d*=0.124), PP (*t*(19) = 0.07; *P*=0.94; *d*=0.117) and HR (*t*(19) = 0.3; *P*=0.77; *d*=0.13) prior to intervention. Prior to intervention night-time SBP, DBP, MAP, PP and HR were also similar prior to intervention (*P*=>0.05; Table 5.1. page 301). Finally, the clinic SBP. DBP, MAP, PP and HR were also similar prior to intervention (*P*=>0.05; Table 5.1. page 301).

5.3.2 Dietary nitrates and nitrites

5.3.2.1 Plasma nitrate and nitrite concentrations

5.3.2.1.1 Baseline

Plasma NO_3^- and NO_2^- were measured in 9 out of 9 of the placebo group (100%) and 9 out of 12 of the dietary NO_3^- group (75%). Baseline plasma NO_3^- were

similar between the placebo (25.58±7.95 μM/L) and dietary NO_3^- group (31.89±11.47 μM/L) (t(16)=1.301; *P*=0.21; *d*=0.64; Figure 5.7; page 315). Similarly, baseline plasma NO_2^- levels were similar between placebo (0.33±0.21 μM/L) and the dietary NO_3^- group (0.23±0.16 μM/L) (t(16)=1.105; *P*=0.29; *d*=0.54; Figure 5.7, page 315).

5.3.2.1.2 Pre-post intervention

As expected, the change in plasma NO_3^- from baseline following 4 weeks of intervention was increased in the dietary NO_3^- group (Δ187.5±120.5 μM/L; 95% confidence interval (CI) [94.9 to 280.1) compared to the placebo group (Δ0.811±11.6 μM/L; 95% CI [-8.1 to 9.7) (t(16)=4.628; *P*=0.0003; *d*=2.18; Figure 5.7; page 315). In addition, the change in plasma NO_2^- concentrations from baseline increased following dietary NO_3^- intervention (Δ0.24±0.29; 95% CI [0.009 to 0.46) compared to the placebo group (Δ-0.1±0.09; 95% CI [-0.18 to -0.03) (t(16)=3.312; *P*=0.004; *d*=1.58; Figure 5.7, page 315). The percentage change in plasma NO_3^- and NO_2^- were also increased following intervention (Figure 5.7, page 315).

5.3.2.2 Resting blood pressure and dietary nitrates

Following 4 weeks of intervention there was no difference in the change in daytime ambulatory SBP between placebo group (3±5 mmHg (95% CI [0 to 6])) and in the active treatment group (3±4 mmHg (95% CI [0 to 5])), (t(19) = 0.28; *P*=0.78, *d*=0.00; Figure 5.8, page 316). There was also no change in daytime ambulatory DBP between the placebo group (0±7 mmHg (95% CI [0 to 7])) and

the active treatment group (0±5 mmHg (95% CI [0 to 5])), (t(19) = 0.22; *P*=0.98, *d*=0.00, Figure 5.8, page 316). No differences were found for daytime ambulatory MAP (*F* (1,19) = 0.439, *P*=0.52; *d*=0.00, Figure 5.8, page 316), daytime ambulatory PP (*F* (1,19) = 3.45, *P*=0.08; *d*=0.00 Figure 5.8, page 316) and daytime ambulatory HR (*F* (1,19) = 1.65, *P*=0.21, *d*=0.00 ; Figure 5.8, page 316). Similar results were found for night-time ABPM for SBP, DBP, MAP, PP and HR (Figure 5.9, page 317).

The change in clinic SBP pre-post treatment was not different between the active treatment group (-11±8 mmHg; 95% CI [-16 to -5]) and placebo intervention (-6±9 mmHg; 95% CI [-13 to 1]), (*t*(19)=1.318, *P*=0.2; *d*=0.59; Figure 5.10, page 318). There was no difference between the active treatment group (-6±5 mmHg; 95% CI [-9 to -3]) and placebo control group (-3±7 mmHg; 95% CI [-9 to 2]) in clinic DBP (*t*(19) = 0.93, *P*=0.37; *d*=0.49, Figure 5.10, page 318). Similar results were found for clinic MAP and PP (Figure 5.10, page 318). In addition, no difference was found in the change in clinic HR between pre and post intervention between the active treatment (-3±10 beats/min; 95% CI [-4 to 5]) and the placebo control group (1±7 beats/min; 95% CI [-11 to 4]) (*t*(19)=1.09, *P*=0.29; *d*=0.46, Figure 5.10, page 318). The antihypertensive medications that participants were taking are shown in Table 5.1 (page 301) and Table 5.3 (page 305). Antihypertensive medication remained the same throughout the study.

5.3.2.3 $\dot{V}O_2$ peak testing

The change in $\dot{V}O_2$ peak pre-post treatment was not different between the active treatment group (0.05±2.34 mmHg; 95% CI [-1.44 to -1.53]) and the placebo intervention group (-0.49±3.12 mmHg; 95% CI [-2.89 to 1.91]) (t(19)=0.45., P=0.66; d=0.2). Similarly, there were no changes in the anaerobic threshold, the peak RER attained, or the peak watts attained during $\dot{V}O_2$ peak testing (P=>0.05, Table 5.4, page 306).

The change in absolute SBP from pre to post intervention at peak exercise ($\dot{V}O_2$ peak testing) was not significantly different between the active treatment group (-5±11 mmHg; 95% CI [-12 to 2]) and the placebo group (4±9 mmHg; 95% CI [-3 to 11]) (t(19) = 0.723; P=0.07; d=0.9; Figure 5.11, page 319). The chance of being beneficial/trivial/harmful (magnitude based inference) was 13/87/0 suggesting that any effect seen for the active group was likely trivial. At peak exercise there was no difference between the pre-post difference in DBP between the active treatment group (2±21 mmHg; 95% CI [-11 to 16]) and placebo control (-1±10 mmHg; 95% CI [-9 to 7]) (t(19) = 0.44; P=0.66; d=0.18; Figure 5.11, page 319). There was no change in MAP from pre to post intervention (t(19) = 1.37; P=0.19; d=0.62) or PP (t(19) = 2.06; P=0.06; d=0.91) at peak exercise (Figure 5.11, page 319). Furthermore, the change in HR from pre to post intervention was not significantly different between the placebo control (-4±6 beats/min; 95% CI [-9 to 0]) and the active treatment group (-1±4 beats/min; 95% CI [-4 to 2]) at peak exercise ($\dot{V}O_2$ peak testing) (t(19) = 1.46; P=0.16; Figure 5.11, page 319).

To assess the absolute and % change from baseline in SBP, DBP, MAP, PP and HR during the incremental exercise test ($\dot{V}O_2$ peak test) a 2-way mixed model ANOVA (within-subject (time; % $\dot{V}O_2$ peak) and between-subject (treatment)) was used. There was not a significant interaction effect for the absolute change in SBP from baseline following the active treatment or placebo during any exercise intensity of $\dot{V}O_2$ peak testing (F (15,190) = 1.15, P=0.32; η^2 = 0.01, Figure 5.13, page 322). Similar to SBP there was no group by time interaction effect for active treatment or the placebo intervention on the absolute change in DBP during any exercise intensity (% of $\dot{V}O_2$ peak testing) (F (15,190) = 0.6, P=0.87; η^2 = 0.02; Figure 5.13, page 322). Similar results were found for the change in MAP, PP or HR during the $\dot{V}O_2$ peak test (Figure 5.13 page 322). Similar results were found for the % change in SBP, DBP, MAP, PP and HR during $\dot{V}O_2$ peak testing (Figure 5.14, page 324).

5.3.2.3.1 Respiratory data

The differences in peak tidal volume, breathing frequency, minute ventilation, V_EVCO_2 (L/min) and the change in RER from pre to post intervention at peak exercise ($\dot{V}O_2$ peak testing) were not significantly different following active treatment when compared to placebo (Figure 5.15, page 326).

The VE/V_EVCO_2 slope during $\dot{V}O_2$ peak testing was not different following active treatment (29.5±4 (pre; 95% CI [27 to 32.1]) vs. 28.4±3 (post; 95% CI [26.5 to 30.4])) or following placebo (29.7±3.1 (pre; 95% CI [27.3 to 32.1]) vs. 28.4±3 (post; 95% CI [28.5 to 33.7]) (F(1,19) = 0.22; P=0.65; η^2 = 0.01). Finally, the rise

in $\dot{V}O_2$ was plotted against the change in watts during $\dot{V}O_2$ peak testing (Figure 5.16, page 328). There was no difference in the rise in $\dot{V}O_2$ during the $\dot{V}O_2$ peak test following dietary NO_3^- or placebo control for 4 weeks ($F(3,38) = 0.32$; $P=0.32$; $\eta^2 = 0.003$, Figure 5.16, page 328). The changes from baseline in respiratory values were not different following NO_3^- or placebo control and can be found in Figure 5.17 to Figure 5.19 (pages 329-333).

5.3.2.4 Resting neural-haemodynamics, handgrip exercise and metaboreflex isolation

5.3.2.4.1 Baseline

The absolute values of MSNA at baseline can be found in Figure 5.20 to 5.22 (pages 335-337). MSNA recordings for baseline, isometric handgrip exercise and PEI were made only in 5 out of 9 (56%) of the placebo group and 8 out of 12 of the dietary NO_3^- group (67%). The change in MSNA burst frequency (bursts/min) during baseline from pre to post treatment was not different following 4 weeks of active treatment (-1±9 bursts/min; 95% CI [-9 to 7]) or placebo (-1±7 bursts/min; 95% CI [-10 to 7]) ($t(11)=0.13$;$P=0.9$;$d=0.00$; Figure 5.23, page 338). The difference in MSNA burst incidence (bursts/100Hb) was also not different following 4 weeks of active treatment (2±19 bursts/100Hb; 95% CI [-14 to 18]) or placebo (-3±10 bursts/100Hb; 95% CI [-16 to 9]) ($t(11)=0.55$;$P=0.59$;$d=0.33$; Figure 5.23, page 338). Total MSNA/time and total MSNA/Hb were assessed in 3 out 5 (60%) of the placebo group and 7 out of 8 of the active group (88%). This was because the active site for MSNA analysis was lost during isometric handgrip or PEI but was put back in for MSNA burst frequency and incidence. As

the distance of the needle from the active site will not be same, the area cannot be calculated. The difference in total MSNA/time and MSNA/Hb were not different following active treatment or placebo (t(8)=0.63; *P*=0.55; *d*=0.46 and t(8)=1.032; *P*=0.33; *d*=0.73; Figure 5.23, page 338).

Spontaneous sympathetic baroreflex sensitivity was measured in all individuals with a baseline MSNA recording. The slope of the spontaneous sympathetic baroreflex sensitivity was similar following active treatment (-2.89±1.77 pre; 95% CI [-4.25 to -1.53] vs -2.35±1.55 post; 95% CI [-3.54 to -1.15] bursts/mmHg) or placebo intervention (-2.45±1.72 pre; 95% CI [-4.59 to -0.31] vs -2.76±1.39 post; 95% CI [-4.49 to -1.1] bursts/mmHg) ($F(1,11) = 0.046$; $P=0.83$; $\eta^2 = 0.004$). Sympathetic vascular transduction was also similar when measured during baseline succeeding active treatment (0.07±0.07 pre; 95% CI [0.02 to 0.12] vs 0.09±0.07 post; 95% CI [0.04 to 0.14] mmHg/%·s) and the placebo (0.09±0.11 pre; 95% CI [0 to 0.24] vs 0.1±0.09 post; 95% CI [0 to 0.21] mmHg/%·s) ($F(1,11)=0.162$; $P=0.7$; $\eta^2 = 0.013$).

5.3.2.4.2 Heart rate variability

All data relating to heart rate variability can be found in Table 5.5 (page 307).

5.3.2.4.2.1 Spectral analysis

No differences were found for either the LF/HF ratio, LF (nu), HF (nu), LF (ms^2) or HF (ms^2) following dietary NO_3^- or placebo in treated controlled hypertension during baseline (*P*=>0.05; Table 5.5, page 307).

5.3.2.4.2.2 Time-domain analysis

No differences were found for either the SDDR (ms), RMSDD (ms) or the pRR50 (%) following dietary NO_3^- or placebo in treated controlled hypertension during baseline (P=>0.05; Table 5.5, page 307).

5.3.2.4.3 Cardiac baroreflex sensitivity

No differences were found for either the overall number of ramps (baroreflex mediated and non-baroreflex ramps), baroreflex mediated ramps, the gain of the cardiac baroreflex or the baroreflex effectiveness index following dietary NO_3^- or placebo in treated controlled hypertension during baseline (*P*=>0.05; Table 5.6, page 308).

5.3.2.5 Isometric handgrip

The difference in absolute SBP from pre to post active treatment (-3±18 mmHg; 95% CI [-15 to 8]) or placebo (9±23 mmHg; 95% CI [-8 to 26]) was not different (t(19)=1.4; *P*=0.18; *d*=0.58; Figure 5.24, page 339). Similarly, there was no difference in absolute DBP from pre to post active treatment (2±9 mmHg; 95% CI [-4 to 8]) or placebo intervention (8±14 mmHg; 95% CI [-3 to 18]) (t(19)=1.14;

P=0.27; *d*=0.51; Figure 5.24, page 339). The change in MAP and PP from pre-post intervention during isometric handgrip was also similar (t(19)=1.3; *P*=0.21; *d*=0.58 and t(19)=0.73; *P*=0.47; d=0.32 Figure 5.24, page 339). The absolute difference in HR during isometric handgrip from pre to post intervention also remained comparable (active treatment 2±4; 95% CI [-1 to 5] vs placebo -1±4; 95% CI [-4 to 2] beats/min; t(19)=0.92; *P*=0.92; d=0.00; Figure 5.25, page 340). No differences in SV (t(19)=0.4; *P*=0.7; d=0.19), CO (t(19)=0.52;P=0.61;d=0.25) or TPR (t(19)=0.97; *P*=0.35; d=0.46) pre to post-intervention (Figure 5.25, page 340).

The change in absolute MSNA burst frequency from pre to post intervention was the same during isometric handgrip exercise following dietary NO_3^- (1±10 bursts/min; 95% CI [-8 to 11]) and placebo (3±3 bursts/min; 95% CI [-2 to 5]) (t(11)=0.04;*P*=0.97; *d*=0.02 Figure 5.26, page 341). In addition, there were no differences in MSNA burst incidence (dietary NO_3^-: 1±17; 95% CI [-15 to 17] vs placebo: 1±5; 95% CI [-2 to 9] bursts/100Hb; t(11)= 0.22; *P*=0.83; *d*=0.14; Figure 5.26, page 341). Similar findings were found for total MSNA/min and total MSNA/Hb (t(8)=0.59; *P*=0.57; *d*=0.43 and t(8)=1.14; *P*=0.29; *d*=0.75; Figure 5.26, page 341).

The absolute change from baseline in SBP during isometric handgrip exercise was similar succeeding active treatment (14±8 pre; 95% CI [9 to 19] vs 12±8 post; 95% CI [6 to 17] mmHg) or following placebo control (11±6 pre; 95% CI [7 to 16] vs 18±8 post; 95% CI [12 to 24] mmHg) (Interaction: *F*(9,108)=0.48;

P=0.89; η^2 = 0.004; Figure 5.29, Page 344). No interaction effect was found for the absolute change in DBP from baseline following the active treatment (7±7 pre; 95% CI [3 to 12] vs 7±5 post; 95% CI [3 to 11] mmHg) or placebo (7±4 pre; 95% CI [4 to 10] vs 10±5 post; 95% CI [7 to 14] mmHg) (*F*(9,108)=0.42; *P*=0.92; η^2 = 0.009; Figure 5.29, Page 344). Comparable results were attained for the absolute change in MAP (F(9,108)=0.36; *P*=0.95; η^2 = 0.005), PP (F(9,108)=0.58; *P*=0.81 η^2 =0.006) or HR (*F*(9,108)=0.68; *P*=0.72; η^2 = 0.02; Figure 5.30, Page 345) from baseline during isometric handgrip exercise (Figure 5.29, Page 344). In addition, no significant interaction was found for the absolute change in SV (*F*(9,108)=0.86; *P*=0.56 η^2 = 0.04), CO (*F*(9,108)=0.96; *P*=0.48; η^2 = 0.04) or TPR (*F*(9,108)=0.08; *P*=0.99; η^2 = 0.003) following active treatment or placebo (Figure 5.30, Page 345). No difference was found for the absolute change in respiratory rate during isometric handgrip testing from pre to post intervention (*P*=>0.05). Similar results were found for the % change in SBP, DBP, MAP, PP, HR, SV, CO, TPR and respiratory rate during isometric handgrip exercise following dietary NO_3^- and placebo intervention (Figure 5.31 and 5.32, Page 346-347).

The change in absolute MSNA burst frequency from baseline during isometric handgrip exercise was similar following active treatment (6±9 pre; 95% CI [-2 to 15] vs 8±7; 95% CI [1 to 14] post bursts/min) and after placebo intervention (7±8 pre; 95% CI [-3 to 17] vs 8±8 post; 95% CI [-3 to 18] bursts/min) (*F*(9,60)=0.45; *P*=0.9; η^2 = 0.03; Figure 5.21, page 336). The change in MSNA burst incidence (bursts/100Hb) from baseline was also similar during isometric handgrip exercise following active treatment (3±13 pre; 95% CI [-9 to 14] vs 6±6 post; 95% CI [1 to 11] bursts/100Hb) and placebo (6±11 pre; 95% CI [-7 to 19] vs 9±10; 95% CI [-4

to 22] post bursts/100Hb) (F(9,60)=0.75; P=0.66; η^2 = 0.04; Figure 5.21, page 336). The absolute change from baseline during isometric handgrip exercise remained similar for total MSNA/min and total MSNA/Hb following dietary NO_3^- and placebo intervention (F(9,48)=0.78;P=0.63; η^2 = 0.03; and F(9,48)=0.59;P=0.8; η^2 = 0.03; Figure 5.21, page 336).

5.3.2.6 Post-exercise ischaemia

The physiological data collected during PEI1 and PEI2 can be found in Figures 5.20 to 5.22 (pages 335-337) and Figures 5.27 to 5.32 (pages 342-347).

The change in absolute SBP during PEI2 from pre to post active treatment (-6±16; 95% CI [-17 to 5] mmHg) or placebo (8±23; 95% CI [-10 to 25] mmHg) was not different (t(19)=1.54; P=0.14; d=0.11; Figure 5.33, page 348). In addition, there was no change in absolute DBP from pre to post active treatment (-1±8; 95% CI [-6 to 5] mmHg) or placebo intervention (6±14; 95% CI [-4 to 17] mmHg) (t(19)=1.41; P=0.17; d=0.61; Figure 5.33, page 348). The absolute change in MAP and PP from pre-post intervention during PEI2 were also not significantly different (t(19)=1.36; P=0.19; d=0.66 and t(19)=1.005; P=0.33; d=0.36; Figure 5.33, page 348). The absolute HR pre to post intervention also remained comparable following active treatment (-2±4; 95% CI [-4 to 1] beats/min) and placebo (2±3; 95% CI [-1 to 5] beats/min) (t(19)=1.64; P=0.0.12; d=0.81; Figure 5.34, page 349). There were no differences in SV (t(19)=1.19; P=0.25; d=0.59), CO (t(19)=0.04;P=0.97;d=0.02) or TPR (t(19)=0.96; P=0.35; d=0.46) pre to post intervention (Figure 5.34, page 349).

During PEI2, the difference in the absolute MSNA burst frequency from pre to post intervention was comparable following active treatment (-4±8; 95% CI [-11 to 3] bursts/min) and placebo (3±5; 95% CI [-3 to 9] bursts/min) (t(11)=0.53;*P*=0.61; *d*=0.33; Figure 5.35, page 350). In addition, there were no differences in MSNA burst incidence from pre to post intervention (active treatment:-3±6; 95% CI [-16 to 17] vs placebo: 2±3; 95% CI [-3 to 6] bursts/100Hb; t(11)=0.32; *P*=0.76; *d*=0.16; Figure 5.35, page 350). Similar findings were found for total MSNA/min and total MSNA/Hb (t(8)=0.84; *P*=0.42; *d*=0.67 and t(8)=0.03; *P*=0.98; *d*=0.02; Figure 5.35, page 350).

The absolute change in SBP during PEI2 from baseline was similar following 4 weeks of active treatment (39±15 pre; 95% CI [29 to 49] vs 35±14 post; 95% CI [25 to 44] mmHg) or following placebo control (36±10 pre; 95% CI [29 to 44] vs 40±15 post; 95% CI [29 to 52] mmHg) (Interaction: *F*(9,108)=0.48; *P*=0.89; η^2 = 0.004; Figure 5.29, page 344). There was no interaction effect for the absolute change in DBP during PEI2 from baseline succeeding active treatment (15±7 pre; 95% CI [10 to 19] vs 12±6 post; 95% CI [8 to 16] mmHg) or placebo (13±3 pre; 95% CI [10 to 15] vs 13±15 post; 95% CI [2 to 24] mmHg) (Interaction: *F*(9,108)=0.42; *P*=0.92; η^2 = 0.009; Figure 5.29, Page 344). Similar results were found for the absolute change in MAP (Interaction: F(9,108)=0.36; *P*=0.95; η^2 = 0.005) and PP (Interaction: F(9,108)=0.58; *P*=0.81 η^2 =0.006) during PEI2 (Figure 5.29, Page 344). No interaction effect was found for the absolute change from baseline in HR during PEI2 after active treatment (2±5 pre; 95% CI [-2 to 5] vs -1±6 post; 95% CI [-5 to 3] beats/min) or placebo (-1±6 pre; 95% CI [-6 to 4] vs 2±5 post; 95% CI [0 to 7] beats/min) (*F*(9,108)=0.68; *P*=0.72; η^2 = 0.02; Figure

5.30, page 345). No significant interaction effects were found for the absolute change in SV ($F(9,108)=0.86$; $P=0.56$ $\eta^2 = 0.04$), CO ($F(9,108)=0.96$; $P=0.48$; $\eta^2 = 0.04$) or TPR ($F(9,108)=0.08$; $P=0.99$; $\eta^2 = 0.003$) during PEI2 following dietary NO_3^- or placebo intervention (Figure 5.30, page 345). There were also no differences in the absolute change in respiratory rate pre or post intervention during PEI2 ($P=>0.05$). Similar results were found for the % change in SBP, DBP, MAP, PP, HR, SV, CO, TPR and the respiratory rate during PEI2 following dietary NO_3^- and placebo intervention (Figures 5.31 and 5.32, page 346-347).

The change in absolute MSNA burst frequency during PEI2 from baseline was similar after active treatment (7±7 pre; 95% CI [1 to 14] vs 6±2 post; 95% CI [4 to 8] bursts/min) and after placebo intervention (8±6 pre; 95% CI [0 to 15] vs 9±7 post; 95% CI [0 to 17] bursts/min) ($F(9,60)=0.45$; $P=0.9$; $\eta^2 = 0.03$; Figures 5.21, page 336). The change in MSNA burst incidence (bursts/100Hb) from baseline was comparable during PEI2 following active treatment (9±16 pre; 95% CI [-6 to 24] vs 11±9 post; 95% CI [3 to 20] bursts/100Hb) and placebo (15±13 pre; 95% CI [-1 to 31] vs 18±14 post; 95% CI [0 to 35] bursts/100Hb) ($F(9,60)=0.75$; $P=0.66$; $\eta^2 = 0.04$; Figures 5.21, page 336). The absolute change in total MSNA/min and total MSNA/Hb from baseline followed a similar pattern following active treatment and placebo intervention ($F(9,48)=0.78$;$P=0.63$; $\eta^2 = 0.03$; and $F(9,48)=0.59$;$P=0.8$; $\eta^2 = 0.03$; Figure 5.21, page 336). An example MSNA trace from baseline to PEI2 can be found in Figure 5.36 (page 351).

5.3.2.7 Recovery

There was no effect of active treatment or placebo intervention for 4 weeks on the absolute, change or % change in SBP, DBP, MAP, PP, HR, SV, CO, TPR, MSNA burst frequency, MSNA burst incidence, MSNA area, total MSNA/min and total MSNA/Hb during recovery from PEI (*P*=>0.05) (Figures 5.37 to 5.45, pages 352-360). A mixed model ANOVA (within-subject (time) and between-subject (treatment)) was used to assess the spontaneous sympathetic baroreflex sensitivity and sympathetic vascular transduction during the 5-minute recovery period. The slope of the spontaneous sympathetic baroreflex sensitivity during recovery was similar following active treatment (-1.16±1.25 pre; 95% CI [-2.31 to -2.71] vs -1.65±1.14 post; 95% CI [-2.71 to -0.6] bursts/mmHg) and placebo intervention (-1.19±1.25 pre; 95% CI [-2.74 to 0.36] vs -1.48±1.18 post; 95% CI [-2.94 to -0.02] bursts/mmHg) ($F(1,10) = 1.87$; $P=0.201$; $\eta^2 = 0.158$). There were also no differences in the spontaneous sympathetic baroreflex sensitivity from baseline to recovery pre or post intervention ($F(3,30) = 4.25$; $P=0.013$; $\eta^2 = 0.298$). Sympathetic vascular transduction was also similar when measured during recovery after active treatment (0.15±0.08 pre; 95% CI [0.08 to 0.23] vs 0.11±0.12 post; 95% CI [0.004 to 0.23] mmHg/%·s) and placebo (0.11±0.17 pre; 95% CI [-0.1 to 0.32] vs 0.15±0.15 post; 95% CI [-0.03 to 0.34] mmHg/%·s) ($F(1,10)=0.00$; $P=0.98$; $\eta^2 = 0.00$). Finally, there were no differences from baseline to recovery pre or post treatment ($F(3,30) = 0.733$; $P=0.54$; $\eta^2 = 0.068$).

5.3.2.7.1 Heart rate variability

5.3.2.7.1.1 Spectral analysis

No differences were found for either the LF/HF ratio, LF (nu), HF (nu), LF (ms^2) or HF (ms^2) following dietary NO_3^- or placebo in treated controlled hypertension during recovery from metaboreflex testing (*P*=>0.05; Table 5.4, page 306).

5.3.2.7.1.2 Time-domain analysis

No differences were found for either the SDDR (ms), RMSDD (ms) or the pRR50 (%) following dietary NO_3^- or placebo in treated controlled hypertension during recovery from metaboreflex testing (*P*=>0.05; Table 5.4, page 306).

5.3.2.7.2 Cardiac baroreflex sensitivity

No differences were found for either the overall number of ramps (baroreflex mediated and non-baroreflex ramps), baroreflex mediated ramps, the gain of the cardiac baroreflex or the baroreflex effectiveness index following dietary NO_3^- or placebo in treated controlled hypertension during recovery from metaboreflex testing (*P*=>0.05; Table 5.5, page 307).

5.4 Discussion

This single-centre, double-blinded, randomised placebo-controlled trial with a parallel group design was the first study to assess the effect of dietary NO_3^- on the BP and MSNA response to metaboreflex and peak exercise testing. The

elevation in plasma NO_3^- and NO_2^- indicates increased dietary NO_3^- intake and suggests an increased substrate pool for endothelial independent NO production is increased in treated controlled hypertensive patients over the intervention period. In contrast to the hypotheses, dietary NO_3^- did not cause a reduction in BP or MSNA during metaboreflex isolation, or a reduction in BP during peak exercise testing.

5.4.1 *Plasma NO_3^- and NO_2^- concentrations*

The current study found that dietary NO_3^- intake for 4 weeks lead to a 667% increase in plasma NO_3^- concentration, which is similar to values reported in previous literature (Kapil et al., 2015, Bondonno et al., 2015). Participants were asked to maintain a normal diet throughout the study and the placebo group's plasma NO_3^- levels did not change over the 4-week period. Furthermore, plasma NO_2^- levels were also elevated by 95% following dietary NO_3^- intervention. This confirms previous findings that the entero-salivary tract is still intact and functioning normally in treated controlled hypertensives (Kapil et al., 2015). Most importantly this suggests that the participants adhered to the beetroot juice over the 4-week period. A previous study in treated and untreated hypertensives found that 4 weeks of dietary NO_3^- caused a significant reduction in clinic, home and daytime ABPM (Kapil et al., 2015). However, in this study there was no effect of dietary NO_3^- intervention on clinic or daytime ABPM. Interestingly, the participants in Kapil et al. (2015) study had treated-uncontrolled BP (daytime ABPM: 139/84 mmHg), whereas the participants in this study were treatment-controlled hypertensives (daytime ABPM: 125/80 mmHg). A previous study assessing

dietary NO_3^- in patients with treated controlled hypertension also found no effect on daytime ABPM, home or clinic BP (Bondonno et al., 2015). The authors postulated that the disparity between their results and those of Kapil et al. (2015) were due to the level of resting BP. This fits with the literature because it has been shown that the acute effect of an antihypertensive medication is proportional to the level of BP at rest (Law et al., 2009). In addition, larger reductions in BP were seen following acute dietary NO_3^- intake when baseline BP was higher (Webb et al., 2008b, Kapil et al., 2010, Ghosh et al., 2013). Antihypertensive medications may also increase the bioavailability of NO in patients with hypertension (Ignarro et al., 2002). Therefore, a change in BP may not have been expected in the already well controlled hypertensives in this study. The lack of improvement in resting BP was associated with no improvements in resting MSNA, or cardiac and sympathetic baroreflex sensitivity following dietary NO_3^- intervention. A similar study, but in healthy individuals found that improvement in resting BP was not associated with an improvement in cardiac baroreflex sensitivity following dietary NO_3^- (Schneider et al., 2018).

5.4.2 $\dot{V}O_2$ peak and the metaboreflex

It was hypothesised that dietary NO_3^- would reduce metaboreflex hyperreflexia and lower MSNA response to PEI in treated controlled hypertension. It was thought that reducing metaboreflex hyperreflexia would help to reduce the blood pressure response to dynamic exercise This is important to assess because the BP response to exercise is an independent risk factor for adverse CV events in the general population (Kurl et al., 2001, Laukkanen et al., 2006). Treated

controlled hypertensives have an exaggerated rise in BP at submaximal and peak exercise ($\dot{V}O_2$ peak testing) similar to untreated and treated uncontrolled hypertensives, which is elevated compared to normotensives (Chant et al., 2018). Of concern, this may place these treated controlled hypertensives at increased CV risk when compared to normotensives (Lawlor et al., 2011). Previous research in healthy young humans has shown that dietary NO_3^- intake dampens the increase in BP during 40, 60 and 80% of $\dot{V}O_2$ peak (Bond et al., 2013). In addition, following 15 days of dietary NO_3^- at a similar dose to this study (5.6 mmol/day) the SBP was reduced at 30 and 60% of $\dot{V}O_2$ peak following compared to a placebo in young (age: 23±1) untreated pre-hypertensive individuals. However, the data presented in this Chapter highlight that the difference in peak SBP following dietary NO_3^- or placebo intervention was not different. In addition, dietary NO_3^- had no effect on BP measured at any submaximal intensity of $\dot{V}O_2$ peak testing. The VE/V_EVCO_2 slope, which is also an independent risk factor for adverse CV events is known to be mediated by the peripheral chemoreceptors and the metaboreceptors (Ponikowski et al., 2001). It has been shown that NO can have an inhibitory effect on the chemoreceptors (Wang et al., 1994). There was no effect of dietary NO_3^- on the VE/V_EVCO_2 slope during $\dot{V}O_2$ peak testing.

Previous research has highlighted the importance of the metaboreflex for mediating exaggerated rises in SBP during exercise in patients with hypertension (Delaney et al., 2010, Sausen et al., 2009). Increases in BP during metaboreflex isolation are mostly mediated by the SNS (Delaney et al., 2010). The hypothesis for this study was that dietary NO_3^- would decrease the BP and MSNA response during metaboreflex isolation (PEI). Only one study has looked at dietary NO_3^- and the metaboreflex. This study found that in healthy older participants (age:

67±2 years) that 4 weeks of dietary NO_3^- reduced the SBP response to PEI compared to a placebo (Schneider et al., 2018). Unfortunately, this latter study did not assess MSNA and the authors postulated that the reduction in BP during PEI was associated with a fall in MSNA (Schneider et al., 2018). One study in young healthy individuals found that acute dietary NO_3^- intervention reduced MSNA at rest and during isometric handgrip exercise (Notay et al., 2017). Notay et al. (2017) suggested that the reductions in MSNA were due to the central effects of NO as the decrease in MSNA was not associated with improvements in sympathetic baroreflex sensitivity. These data suggest that dietary NO_3^- can inhibit sympathetic outflow from the brainstem (Notay et al., 2017). This study found no effect of dietary NO_3^- intervention for 4 weeks on MSNA during resting, isometric handgrip, PEI or recovery. Interestingly, a recent study found that in healthy individuals (aged between 18-48 years) that isolation of the metaboreflex only leads to increases in MSNA in the non-contracting limb, not the contracting limb (Boulton et al., 2018). MSNA was measured in the non-contracting right leg in this study and future research will need to assess the findings of Boulton et al. (2018) in individuals with hypertension. SBP also remained the same at baseline and during isometric handgrip and PEI following the intervention period. The following section focuses on plausible mechanisms for the lack of effect of dietary NO_3^- to improve the neural-haemodynamic response to peak exercise testing ($\dot{V}O_2$ peak testing) and metaboreflex isolation (PEI).

5.4.3 *Putative mechanisms for the lack of effect of dietary NO_3^- during exercise or metaboreflex isolation*

There are several plausible mechanisms why elevated levels of NO_3^- and NO_2^- did not suppress the CV reactions during exercise in the treated-controlled hypertensives in this study. Firstly, angiotensin II and ROS (such as O_2^-) increase in the skeletal muscle in relation to exercise intensity in healthy individuals (Moralez et al., 2018, Bailey et al., 2003). NO is quickly scavenged by O_2^-, forming the oxidant peroxynitrite (Pattwell et al., 2004). O_2^- and peroxynitrite can oxidise guanylyl cyclase making it unresponsive to NO (Stasch et al., 2006). In healthy Sprague-Dawley rats the increase in ROS during hindlimb contraction is modest, whereas in angiotensin II infused hypertensive rats the increase is substantially elevated (Zhao et al., 2006). This increase in O_2^- decreased functional sympatholysis and increased the BP response to exercise in the hypertensive rats (Zhao et al., 2006). It could be speculated that irrespective of how NO is increased it will have little effect on functional sympatholysis and subsequently the metaboreflex in hypertensive individuals, unless ROS levels are depressed (Zhao et al., 2006). The improvement in functional sympatholysis and the BP response to dynamic handgrip exercise testing following 12 weeks of nebivolol may reflect nebivolols antioxidant capabilities (Price et al., 2013). Indeed, nebivolol has been shown to reduce the subunits Rac1 and $p67^{phox}$ which are necessary for the oxidative activity of nicotinamide-adenine dinucleotide phosphate oxidase (NAD(P)H) (Oelze et al., 2006, Whaley-Connell et al., 2009). Through this mechanism it is proposed that nebivolol reduces exercise induced increases in in O_2^- (Oelze et al., 2006). Nebivolol also stimulates the release of NO by binding to endothelial β_2 and β_3 adrenergic receptors (Broeders et al.,

2000, Dessy et al., 2005) in addition to the stimulation of purinergic receptors (P2Y receptors) by endothelial ATP efflux (Kalinowski et al., 2003). Furthermore, tetramethyl-pereride 1-oxyl (Tempol), a known scavenger of O_2^- improves functional sympatholysis and exercise BP in the active hindlimb during contraction in angiotensin II treated hypertensive animals (Zhao et al., 2006). As β-adrenoceptor antagonists are known to impair exercise capacity (Van Baak, 1988), drugs that target reactive O_2 species and that increase NO, whilst improving exercise capacity may be more beneficial in hypertension.

There is considerable evidence that NO plays an important role in functional sympatholysis and BP regulation during exercise (Price et al., 2013, Thomas and Victor, 1998). However, a significant body of research is overlooked which suggests that NO is not obligatory for either functional sympatholysis or BP control during exercise (Rosenmeier et al., 2003, Campbell et al., 2011, Radegran and Saltin, 1999). Firstly, acute local NO inhibition with N(G)-monomethyl-L-arginine (L-NMMA) increased BP at rest and during sub-maximal exercise, but not at maximal exercise in healthy subjects (Campbell et al., 2011). In addition, L-NMMA has no effect on femoral blood flow or BP during submaximal or maximal, one-legged, dynamic knee-extensor exercise (Radegran and Saltin, 1999). Finally, exogenous NO stimulation does not blunt sympathetic vasoconstriction in healthy humans (Rosenmeier et al., 2003). Furthermore, it has been shown that NO works in synergy with other substances, such as prostacyclin, to mediate functional sympatholysis (Casey and Joyner, 2011, Dinenno and Joyner, 2004). However, the above-mentioned studies were conducted in healthy individuals and the available data in hypertensive

individuals does suggest an important role of NO (Price et al., 2013, Thomas and Victor, 1998). Nevertheless, the results presented in this Chapter suggest a non-obligatory role of NO during exercise in treated-controlled hypertension.

5.4.4 Limitations

Firstly, due to time constraints in completing this thesis the study had to be stopped prematurely meaning that the study was potentially left underpowered compared to other studies to find any differences in the primary outcome variable. The original power calculations suggested that 40 participants were needed for this study and only 21 were recruited. Future research will need to reconfirm these findings with a larger sample size. Plasma NO_3^- and NO_2^- concentrations were measured at rest in this study and it was assumed that NO_3^- and NO_2^- levels remained elevated during exercise in this group. An interesting study in healthy adult Wistar rats found that an acute bout of exercise caused a reduction in blood NO_3^- and NO_2^-, which indicated increased NO formation in the skeletal muscle (Piknova et al., 2016). This is important to assess in hypertension because maintained levels of NO_3^- and NO_2^- during exercise would suggest that the reductase mechanisms that reduce NO_2^- to NO are dysfunctional during exercise in hypertension. However, previous research in spontaneous hypertensive rats at rest has demonstrated that xanthine oxidoreductase expression is increased, suggesting an enhanced ability to reduce NO_2^- to NO (Ghosh et al., 2013). Cyclic guanosine monophosphate (cGMP), the most sensitive marker of increase NO activity was not measured in the participants in this study (Ghosh et al., 2013, Kapil et al., 2015). Previous research has found

that plasma cGMP is increased in a temporal pattern following dietary NO_3^- intake at rest (Kapil et al., 2015, Kapil et al., 2010). The previous literature in treated hypertensive individuals that has also shown a temporal increase in plasma cGMP following dietary NO_3^- consumption at rest (Kapil et al., 2015). Future studies should measure cGMP during exercise in hypertension to confirm the adequate reduction of NO_2^- to NO. In addition, the dose of dietary NO_3^- used in this study was similar to previous research that found that ABPM was reduced following dietary NO_3^- consumption (Kapil et al., 2015). Whether a larger dose of dietary NO_3^- is needed to have an effect during exercise in hypertension is unknown and requires further research. Furthermore, it was assumed that elevated ROS levels inhibited the effect of elevated NO_2^- in this study. Future research will need to measure the level of superoxide anions during exercise, for example using electron spin resonance spectroscopy from venous blood samples (Moralez et al., 2018).

Adherence to anti-hypertensive medication is typically poor (Herttua et al., 2013, Ong et al., 2007) and it is unclear whether poor adherence to the intervention in this study influenced the study results. Although, the plasma NO_3^- and NO_2^- concentrations were elevated in this following dietary NO_3^- for 4 weeks, it cannot be guaranteed that participants were taking the intervention every day. For example, acute dietary NO_3^- consumption increases plasma NO_3^- and NO_2^- (Kapil et al., 2010). A large proportion of participants complained of a foul taste and this likely would likely influence adherence. However, participants did complete a diary which suggested all of them adhered to the interventions.

In addition, the studies that have assessed functional sympatholysis in hypertension have measured BP, MSNA and forearm blood flow response to dynamic handgrip testing (Price et al., 2013, Vongpatanasin et al., 2011). During isometric handgrip exercise, the arterial BP response is much larger as the BP needs to overcome the compressed blood vessels caused by intramuscular pressure increases (Kaur and Mann, 2016, Lind et al., 1964). Skeletal muscle blood flow was not measured in this study and future studies should measure forearm blood flow during dynamic handgrip exercise to assess functional sympatholysis (Price et al., 2013, Vongpatanasin et al., 2011). In addition, Kapil et al. (2015) found the flow-mediated dilatation (FMD) was increased following dietary NO_3^- consumption. As decreased FMD is related to excessive exercise BPs (Stewart et al., 2004), it is important to assess whether improvements in FMD associated with dietary NO_3^- consumption (Kapil et al., 2013, Kapil et al., 2015) are associated with improvements in exercise BP. Finally, only 1 minute of isometric handgrip exercise was used in this study, Delaney et al. (2010) used 90s of isometric handgrip due to difficulties with getting participants to maintain 40% of MVC for longer than 90 seconds. In order to maintain adequate quality of the MSNA signal 1 minute was chosen instead of 90s. Although BP and MSNA did increase, it could be argued that this was not a long enough stimulus to stimulate the metaboreceptors (Crisafulli et al., 2006).

5.4.5 Clinical perspectives

Dietary NO_3^- consumption has previously been shown to lower clinic, home and 24-hour ambulatory BP monitoring in treated and untreated hypertension (Kapil et

al., 2015) This is important as this would be expected to lower CV risk in hypertensive individuals (Lewington et al., 2002, Wright et al., 2015). Unfortunately, dietary NO_3^- consumption for 4 weeks is not associated with improvements resting BP, the BP response to metaboreflex isolation, submaximal exercise (51-75% $\dot{V}O_2$ peak) or peak exercise in treated controlled hypertension. Future research needs to consider different therapeutic strategies for improving the BP response to exercise as an excessive BP response to exercise is an independent risk factor for hypertension (Kurl et al., 2001, Laukkanen et al., 2006).

5.5 Conclusions

This is the first study to assess the effect of consumption of dietary NO_3^- consumption for 4 weeks on the BP and MSNA response to metaboreflex isolation and the BP response to peak exercise testing ($\dot{V}O_2$ peak test). Dietary NO_3^- had no effect on BP or MSNA response to metaboreflex isolation. In addition, there was no effect on the BP response to peak exercise in treated controlled hypertension.

5.6 Tables

Table 5-1 Participant demographics at pre-treatment

Participant demographics	**Placebo (pre)**	**Dietary nitrates (pre)**
N	9	12
M/F	4/5	6/6
Age (Years)	63±8	63±7
Height (cm)	170±7	171±8
Weight (kg)	76±11	76±10
BMI (kg/m^2)	26.7±3	26.2±3
$\dot{V}O_2$ peak (ml/min/kg)	23.8±6.6	25.9±9.9
AT (%)	67.6±6.9	70.4±6.4
Daytime ABPM		
SBP (mmHg)	124±8	125±8
DBP (mmHg)	79±11	80±7
MAP (mmHg)	94±9	95±7
PP (mmHg)	45±11	46±5
HR (beats/min)	64±7	66±10
Night-time ABPM		
SBP (mmHg)	120±15	118±10
DBP (mmHg)	72±10	72±8
MAP (mmHg)	88±11	87±8
PP (mmHg)	48±9	47±5
HR (beats/min)	59±8	62±11
Clinic BP		
SBP (mmHg)	142±13	136±18
DBP (mmHg)	81±9	78±11
MAP (mmHg)	101±8	97±12
PP (mmHg)	61±12	58±13
HR (beats/min)	67±10	63±6
Antihypertensive medications		
Median number of anti-hypertensive medications	2 (IQR=1-2.5)	2 (IQR=1-2)

Percentage of participants taking anti-hypertensives (by class)		
ACEi (%)	44	42
ARB (%)	22	8
CCB (%)	67	67
α-blocker (%)	0	8
β-blocker (%)	11	17
Diuretics (%)	22	8

N; number, M; male, F; female, BMI; body mass index, $\dot{V}O_2$ peak; peak volume of oxygen inspired, AT; anaerobic threshold (%), ABPM; ambulatory blood pressure monitoring, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, PP; pulse pressure, HR; heart rate, ACEi; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, CCB; calcium channel blocker, IQR; inter-quartile range.

Table 5-2 Schedule of Assessments and Procedures

	Pre-assessment 1 (week 1)	**Pre-assessment 2 (week 1)**	**Post-assessment 1 (week 5)**	**Post-assessment 2 (week 5)**
Procedure/Assessment				
Written informed consent	X			
Demographics (Medical history, age, height, weight, BMI)	X	X		X
Vital signs (heart rate, blood pressure and oxygen saturations).	X	X		X
Ambulatory blood pressure monitoring (ABPM) (24 hours)	X		X	
ECG (12-lead) for screening at rest and exercise testing	X	X		X
Urine dipstick test	X			
Blood sample for nitrate and nitrite analysis		X		X
ECG (3-lead), heart rate, oxygen saturation, spirometry (tidal volume, respiratory frequency), partial pressures		X		X

of inspired and expired gases (CO_2 and O_2)				
Microneurography		X		X
Isometric handgrip testing		X		X
Finapres continuous blood pressure measurement (finger cuff)		X		X
Peak exercise testing ($\dot{V}O_2$ peak test)		X		X
Participant given inorganic nitrate supplement or placebo		X		

Table 5-3 Anti-hypertensive medications

Placebo		
Anti-hypertensive class	Specific drug taken	% taking drug
ARB	Losartan	11
	Candesartan	11
ACEi	Ramipril	44
CCB	Amlodipine	56
β-blocker	Bisoprolol	11
Diuretics	Bendroflumethiazide	22
Dietary nitrate		
ACEi	Ramipril	25
	Perindopril	8
	Lisinopril	17
CCB	Amlodipine	58
β-blocker	Atenolol	8
	Bisoprolol	8
α-blocker	Doxazosin	8
Diuretics	Bendroflumethiazide	8

ACEi; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, CCB; calcium channel blocker

Table 5-4 Pre- and post-treatment $\dot{V}O2$ peak data

	Placebo		**Dietary nitrates**	
	Pre	**Post**	**Pre**	**Post**
$\dot{V}O_2$ peak	23.8±6.6	23.3±6.9	25.9±9.9	26±10.8
AT (%)	67.6±6.9	66.9±9	72.5±5.3	69.2±7.9
RER_{MAX}	1.36±0.07	1.32±0.06	1.27±0.06	1.26±0.1
$Watt_{MAX}$	174±50	175±55	200±83	196±82

$\dot{V}O_2$ peak; peak volume of oxygen inspired, AT; anaerobic threshold, RER; respiratory exchange ratio.

Table 5-5 Heart rate variability (HRV).

Heart rate variability (HRV)								
Spectral analysis								
	Baseline				**Recovery**			
	Placebo Pre	**Placebo Post**	**Dietary nitrates Pre**	**Dietary nitrates Post**	**Placebo Pre**	**Placebo Post**	**Dietary nitrates Pre**	**Dietary nitrates Post**
LF/HF ratio	1.25±0.6	1.35±0.9	1.99±2.7	1.63±1.1	1.91±1.17	2.09±1.92	1.59±0.96	1.22±1.13
LF (nu)	48.32±15.8	47.26±17.9	48.49±24.1	50.62±22.1	58.03±14.75	56.78±18.97	52.92±18.75	43.77±20.76
HF (nu)	41.88±11.44	43.73±15.45	42.07±20.08	44.7±20.3	37.33±13.04	39.1±16.59	42.51±19.22	48.91±18.29
LF (ms^2)	434.64±257.6	427.48±244.6	499.06±533.2	529.98±509.2	818.8±578.21	674.48±489.16	917.93±907.63	711.81±444.66
HF (ms^2)	607.31±390.7	592.86±395.7	352.86±307.9	352.64±268.6	418.45±276.82	431.78±257.64	551.77±417.56	682.28±747.89
Time domain								
SDRR (ms)	46.83±15.17	48.83±11.49	0.36±14.84	43.77±18.82	54.61±19.44	53.99±14.8	76.91±47	47.82±16.59
RMSSD (ms)	34.17±14.32	41.19±14.32	34.24±16.5	37.65±25.08	37.28±15.92	39.47±14.61	47.49±34.27	38.88±21.93
pRR50 (%)	13.53±12.99	15.8±12.3	11.17±11.61	16.05±19.1	16.05±14.31	16.49±12.14	16.44±11.23	18.66±19.08

LF/HF; low frequency/high frequency ratio, LF (nu); low frequency, HF (nu); high frequency, SDRR; standard deviation of the R-R interval, RMSSD, root mean square of the successive differences, pRR50, percentage of R-R intervals > 50 ms.

Table 5-6 Spontaneous cardiac baroreflex sensitivity.

Spontaneous cardiac baroreflex sensitivity								
Sequence technique								
	Baseline				**Recovery**			
	Placebo		**Dietary nitrates**		**Placebo**		**Dietary nitrates**	
Total with sequences (n)	7/9(78%)		12/12(100%)		7/9(78%)		10/12(83%)	
Up ramps (n)	47±20	43±12	53±26	45±20	27±9	27±10	24±7	25±10
Down ramps (n)	52±15	54±22	59±22	48±20	29±6	30±7	30±10	28±10
All ramps (n)	98±35	97±30	112±46	92±39	56±15	57±16	54±15	52±18
Sequence ramps up (n)	11±11	8±10	7±9	9±9	6±3	3±2	5±5	5±3
Sequence ramps down (n)	18±16	12±13	7±8	10±10	10±4	8±5	5±4	4±4
Sequence ramps all (n)	29±27	20±23	14±16	19±19	16±7	11±6	11±9	9±6
Up ramps (gain)	7.6±3.7	7.6±3.2	10.9±8.1	9.8±5.7	11±8.7	9.7±3	10±6.3	12.16±6.4
Down ramps (gain)	11.5±3.1	9.6±5.4	9.2±4.9	10.1±5	9.9±4.7	10.3±4.5	9±3.2	10.1±3.6
All ramps (gain)	10.1±3.2	8.9±3.9	9.9±4.9	10.2±5.3	9.9±5.5	10±3.7	9.1±3.9	10.5±5
Baroreflex effectiveness index (BEI)								
Total number of participants	7/9(78%)	N/A	12/12(100%)	N/A	7/9(78%)	N/A	10/12(83%)	N/A
BEI (up)	0.23±0.14	0.2±0.22	0.19±0.17	0.23±0.18	0.24±0.07	0.13±0.02*	0.26±0.18	0.19±0.06*
BEI (down)	0.30±0.26	0.24±0.28	0.15±0.18	0.21±0.18	0.32±0.11	0.24±0.12	0.22±0.16	0.19±0.16
BEI (all)	0.25±0.18	0.20±0.25	0.15±0.17	0.21±0.17	0.27±0.09	0.18±0.08	0.21±0.17	0.17±0.1

* denotes a significant difference from pre to post (placebo or dietary nitrates).

5.7 Figures

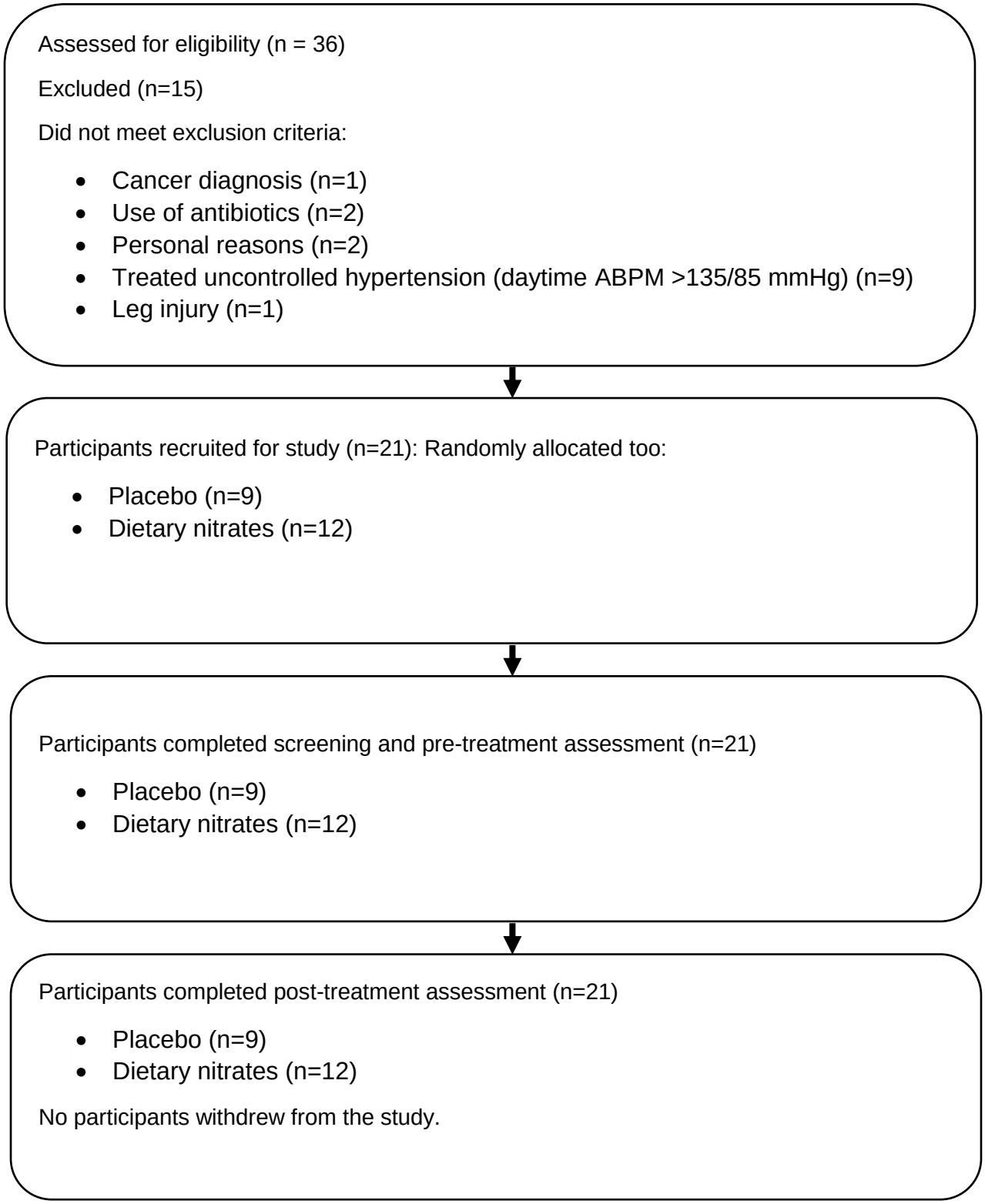


Figure 5-1 Participant screening and recruitment information

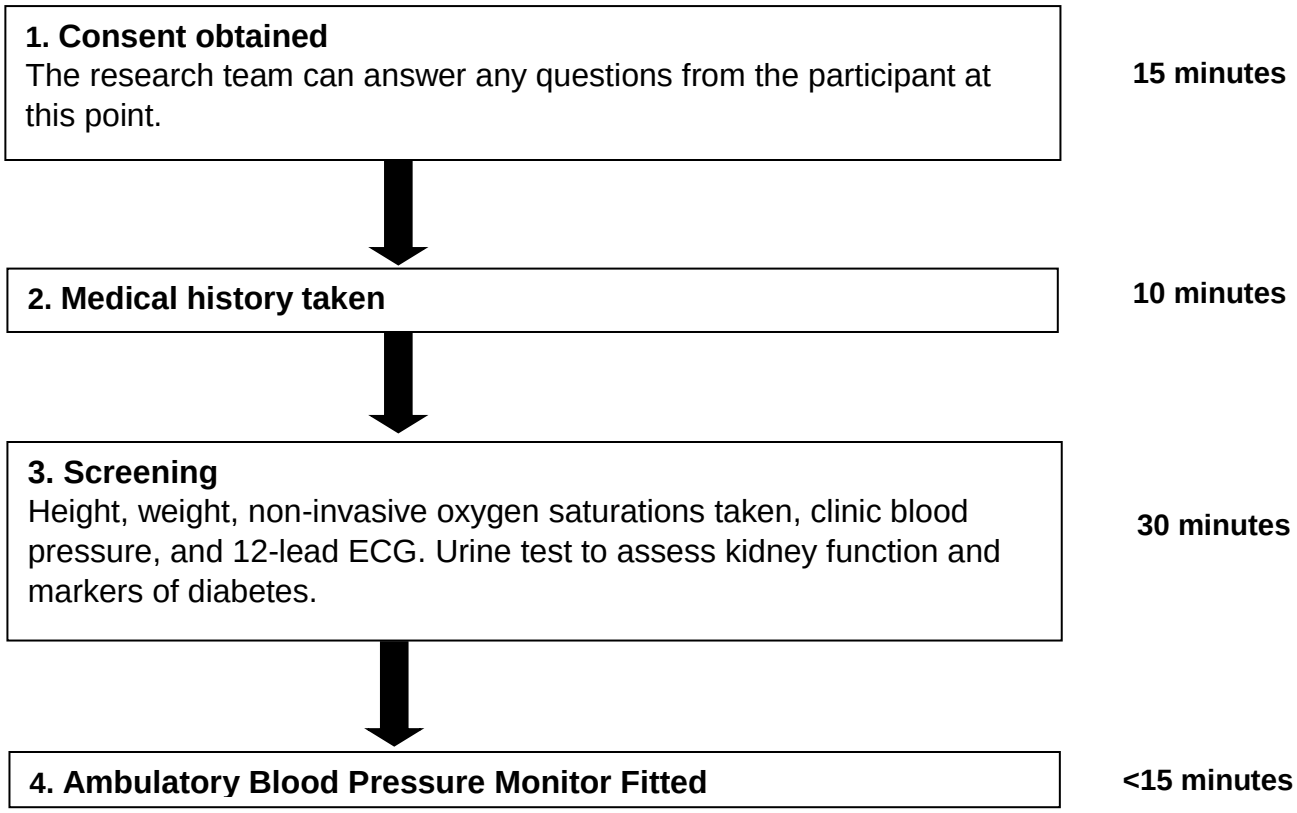


Figure 5-2 Flow Chart for pre-assessment one

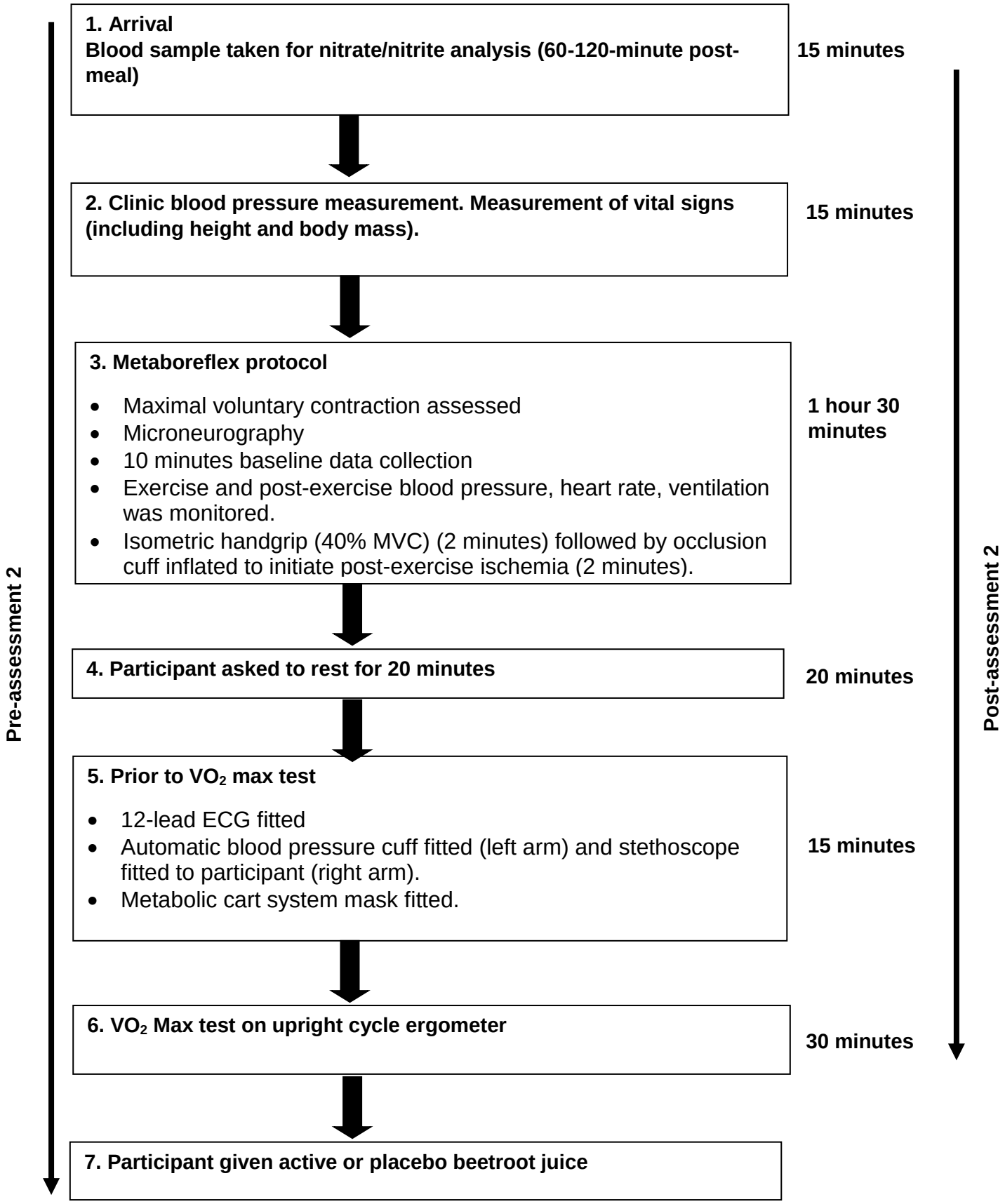


Figure 5-3 Flow chart for pre- and post-assessment. Total Time: ~3 hours

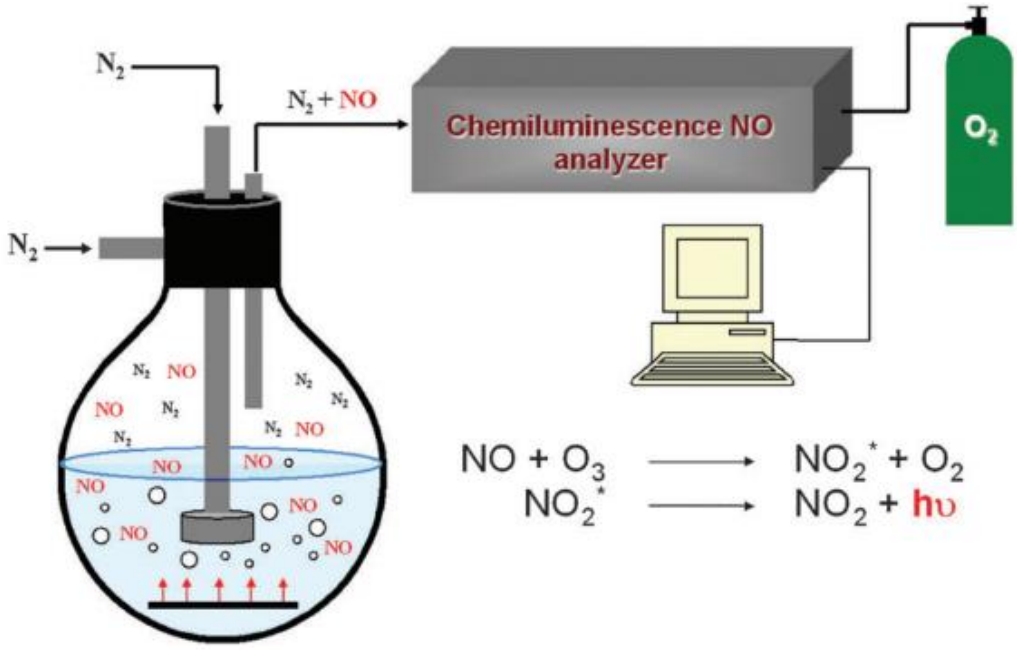


Figure 5-4 Basic set-up for Ozone (O3) based chemiluminescence (image from Coneski and Schoenfisch (2012)).

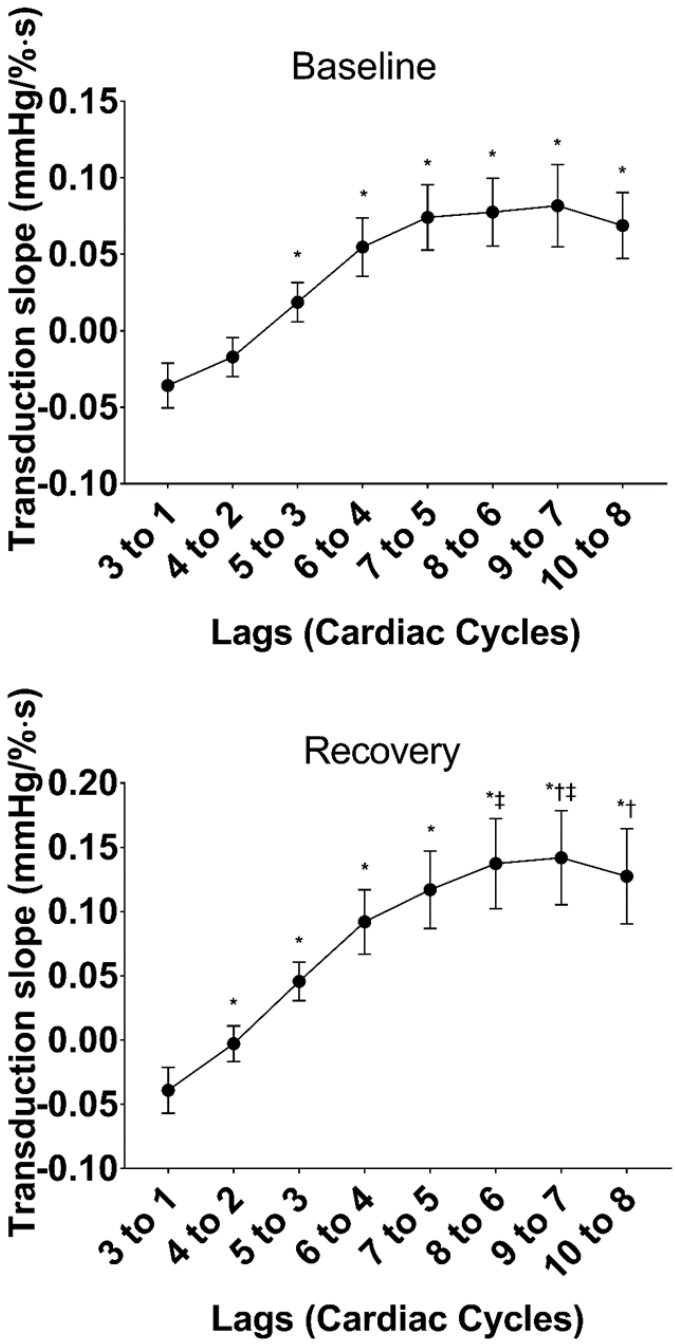


Figure 5-5 Determination of the optimal lag for transduction analysis.

To determine the optimal cardiac cycle lag for treated controlled hypertension a transduction slope was produced for each participant using 8 windows of 2 cardiac cycles. Similar to Briant et al. (2016) 8-6 cardiac lags was used for transduction analysis.

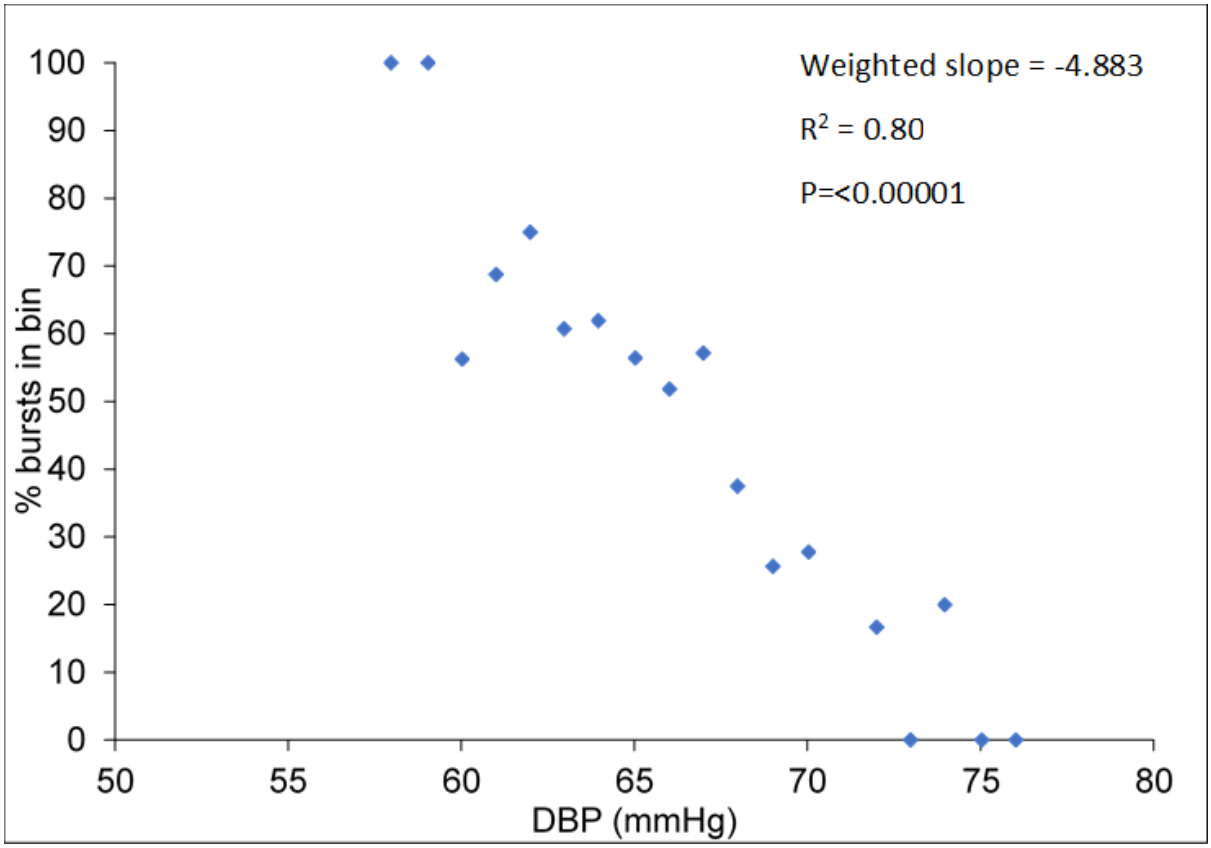


Figure 5-6 An example of the spontaneous baroreflex slope calculated from the 10-minute baseline period.

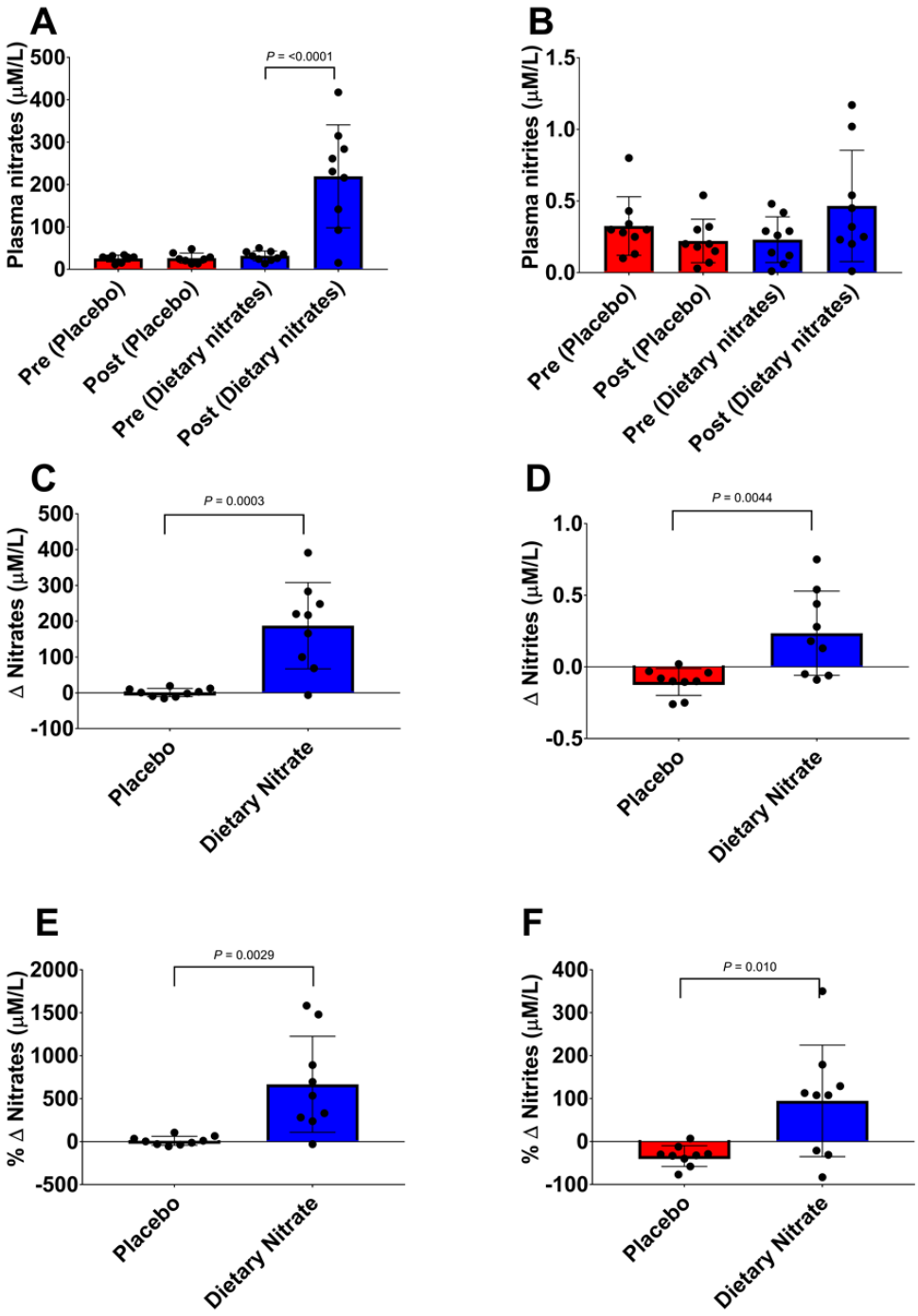


Figure 5-7 Pre and post plasma dietary nitrates and nitrite concentrations.

A) plasma nitrates, B) plasma nitrites, C) the change in plasma nitrates from baseline, D) the change in plasma nitrites from baseline, E) % change in plasma nitrates from baseline and F) % change in plasma nitrites from baseline.

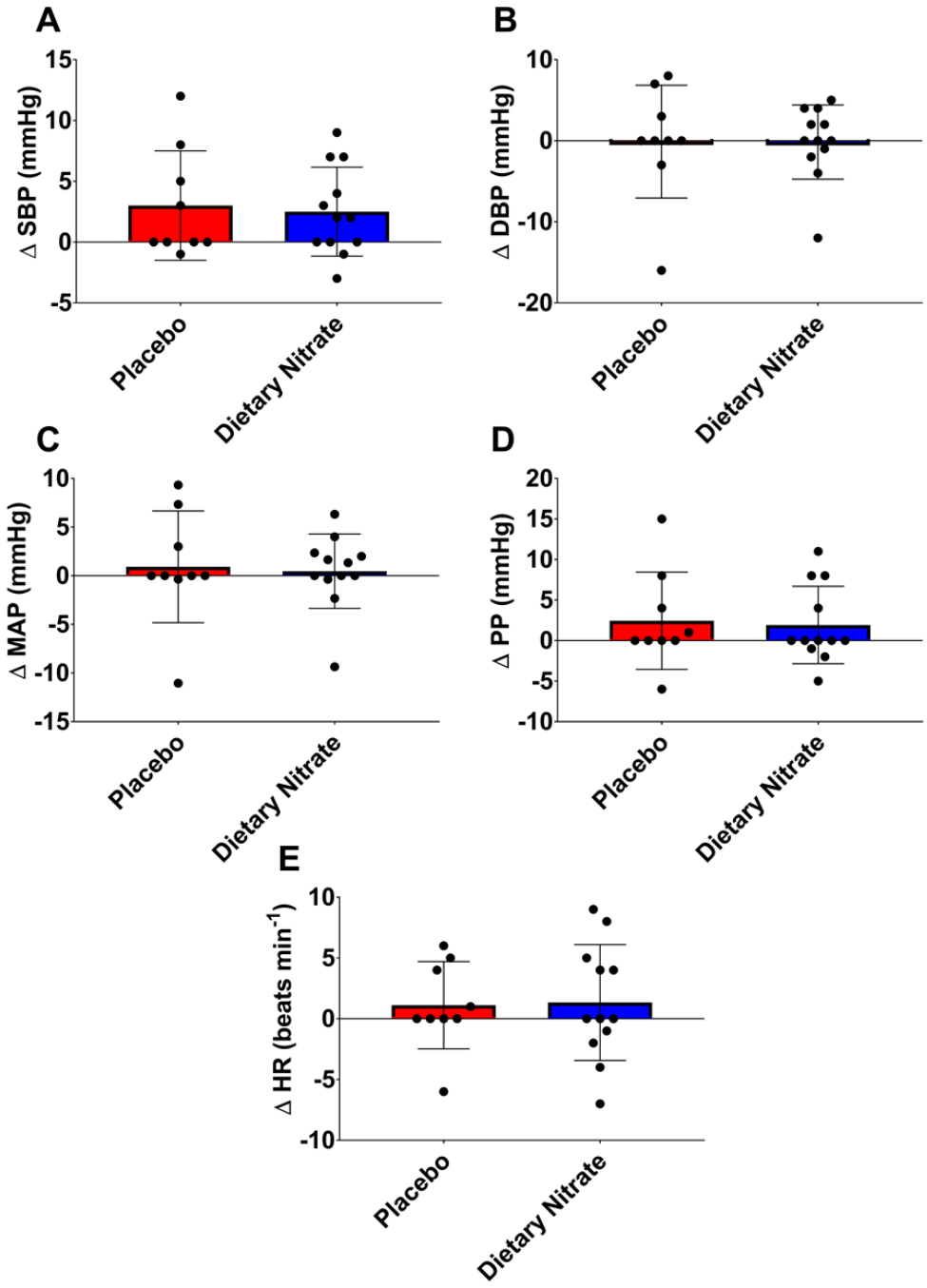


Figure 5-8 The effect of dietary nitrate or placebo on the change in ambulatory daytime haemodynamic measurements in treated controlled hypertension.

The change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) heart rate (HR) from pre to post intervention.

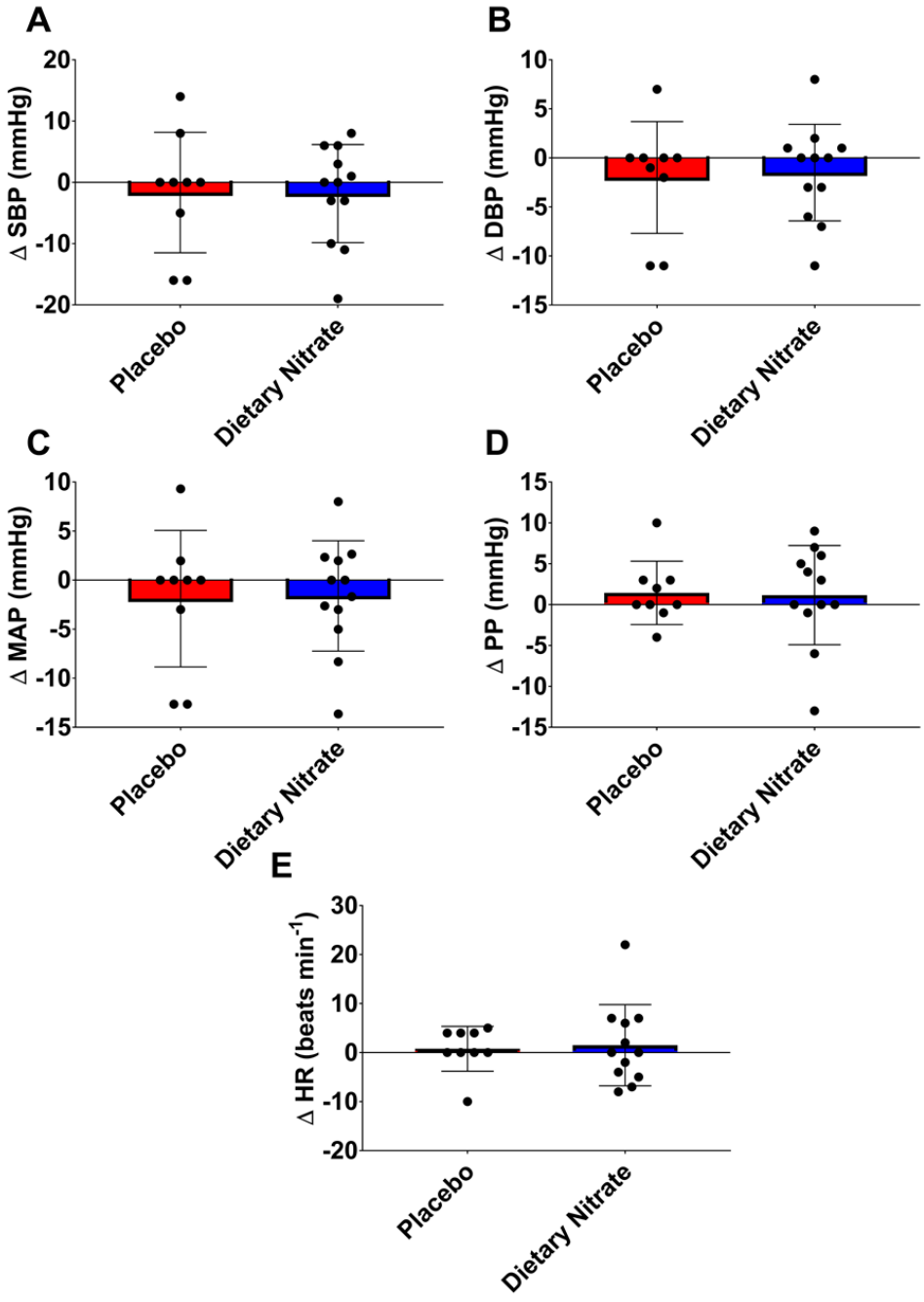


Figure 5-9 The effect of dietary nitrate or placebo on the change in ambulatory night-time haemodynamic measurements in treated controlled hypertension.

The change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) heart rate (HR) from pre to post intervention.

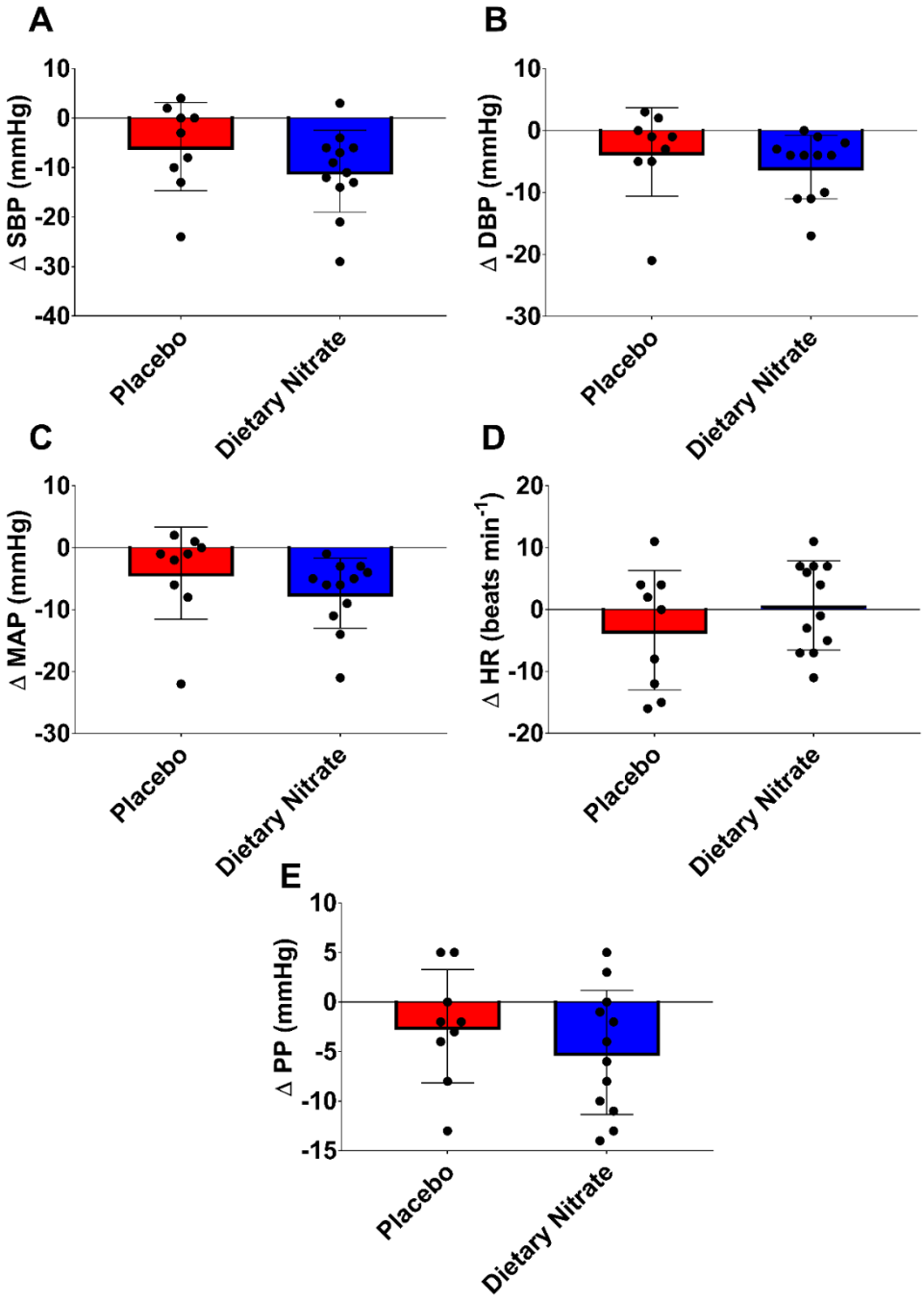


Figure 5-10 The effect of dietary nitrate or placebo on the change in clinic haemodynamic measurements in treated controlled hypertension.

The change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) heart rate (HR) from pre to post intervention.

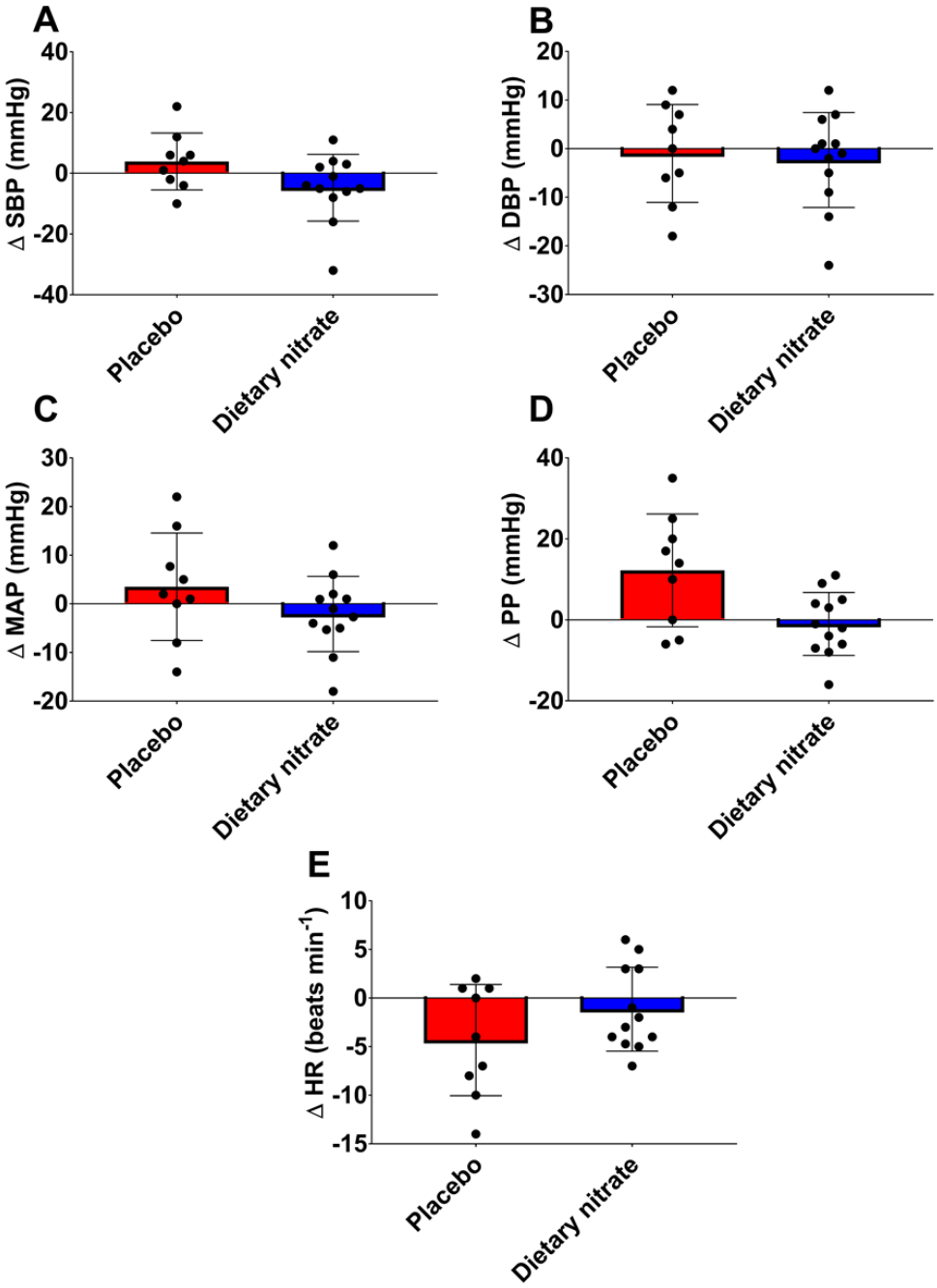


Figure 5-11 The effect of dietary nitrate or placebo on the change in peak haemodynamic measurements during peak cycle ergometer exercise testing

The change in peak A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) heart rate (HR) from pre to post intervention from a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test).

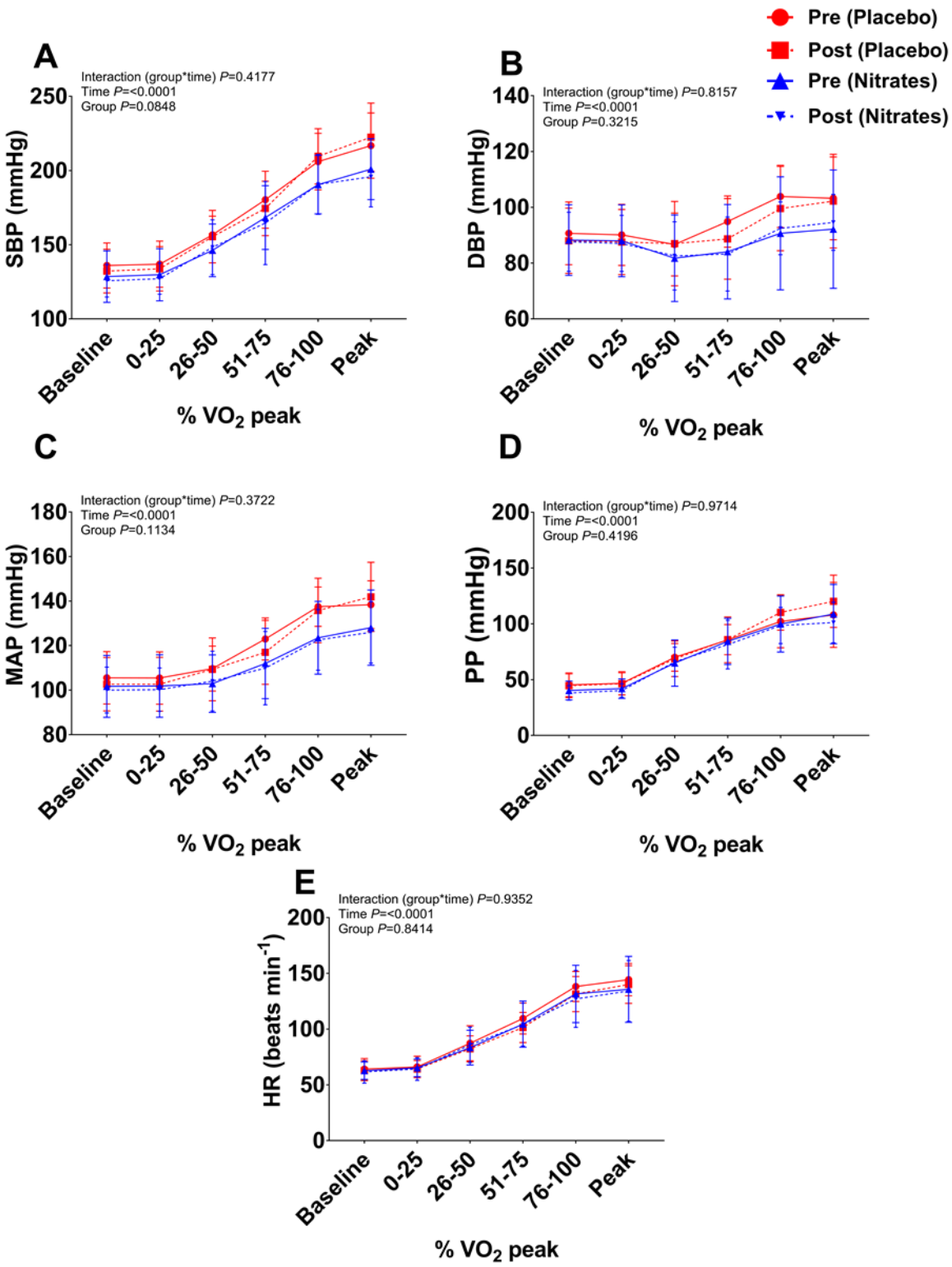


Figure 5-12 The effect of dietary nitrate or placebo on the absolute haemodynamic measurements during peak cycle ergometer exercise testing ($\dot{V}O_2$ peak test).

The absolute A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) heart rate (HR) during a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

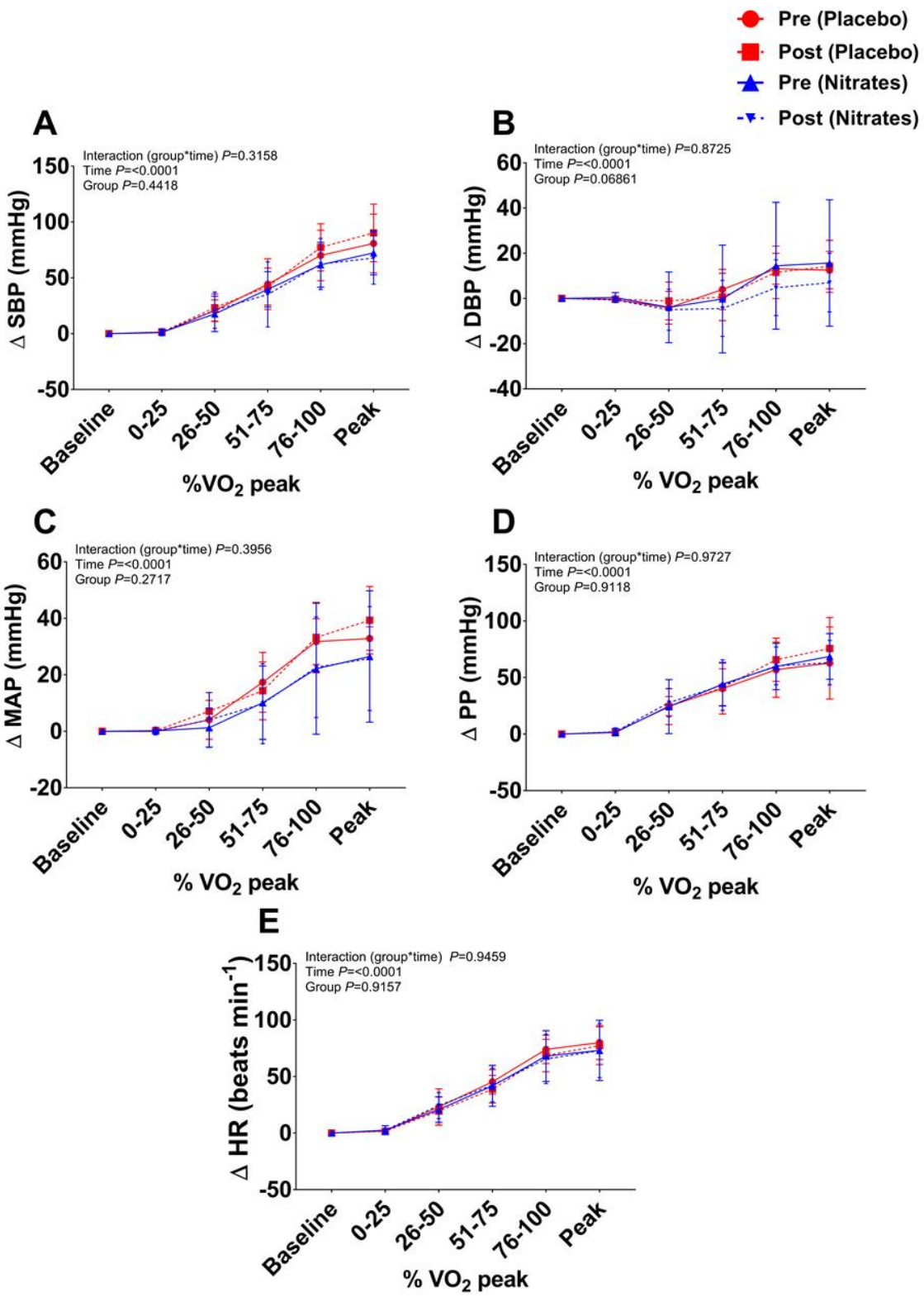


Figure 5-13 The effect of dietary nitrate or placebo on the change in haemodynamic measurements during peak cycle ergometer exercise testing ($\dot{V}O_2$ peak test).

The absolute change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) heart rate (HR) from baseline during a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

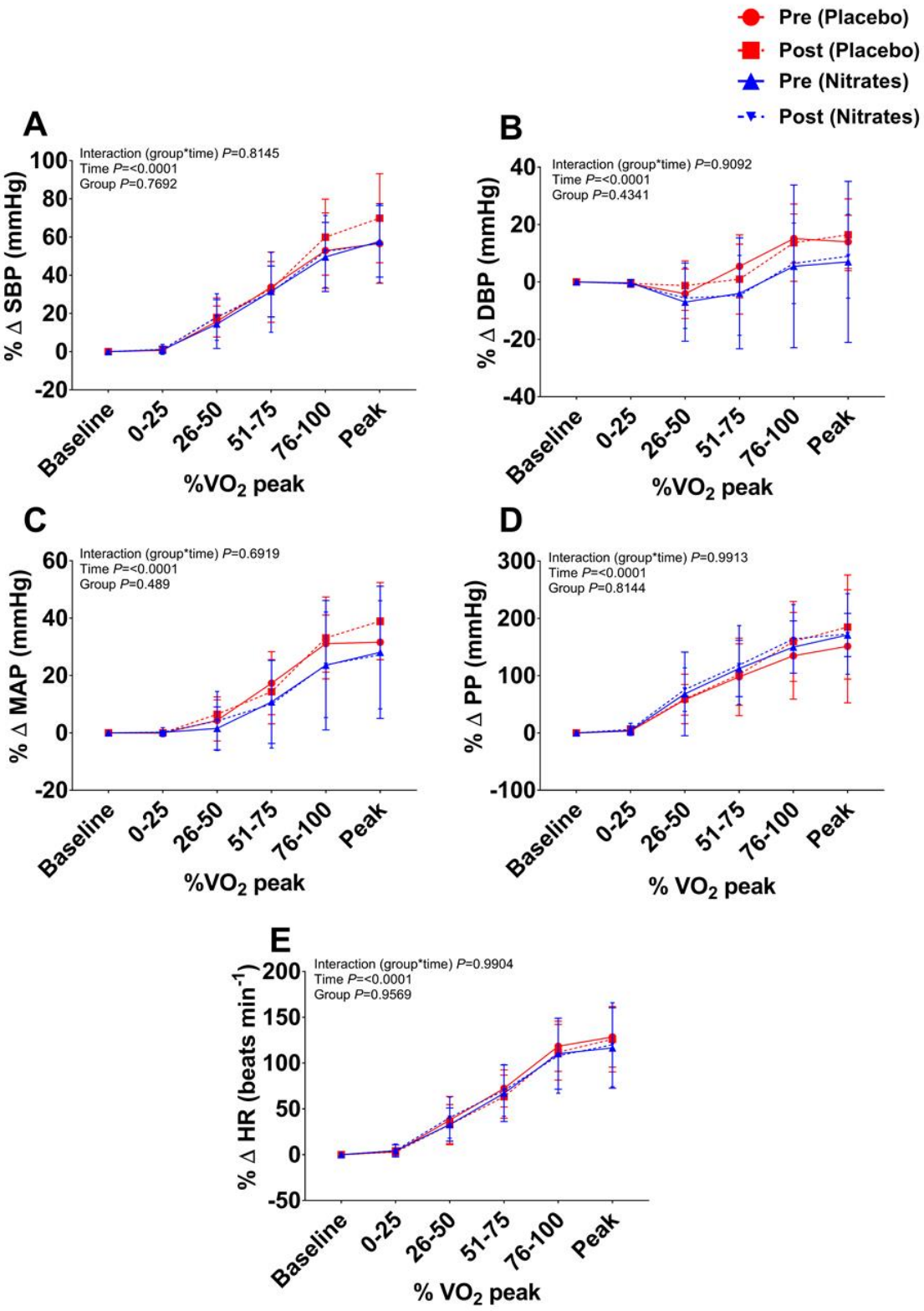


Figure 5-14 The effect of dietary nitrate or placebo on % change in haemodynamic measurements during cycle ergometer exercise testing ($\dot{V}O_2$ peak test).

The percentage (%) change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP), D) pulse pressure (PP) and E) heart rate (HR) from baseline during a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

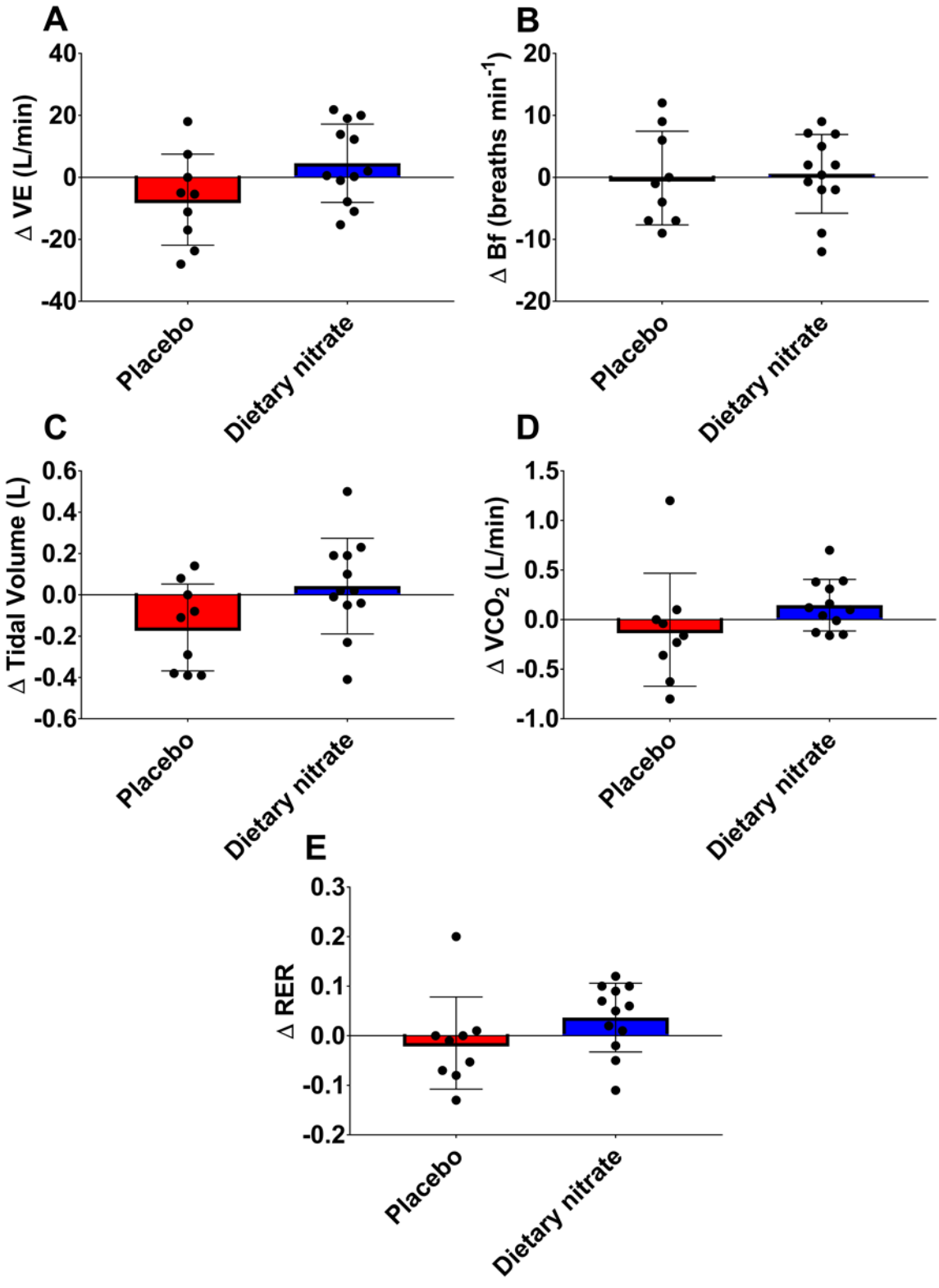


Figure 5-15 The effect of dietary nitrate or placebo on the change in peak respiratory measurements during peak cycle ergometer exercise testing.

The effect of 4 weeks of dietary nitrate or placebo on the change in peak A) minute ventilation (VE), B) breathing frequency (Bf), C) tidal volume, D) volume of carbon dioxide expired (VCO_2) and E) respiratory exchange ratio (RER) from pre to post intervention from a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test).

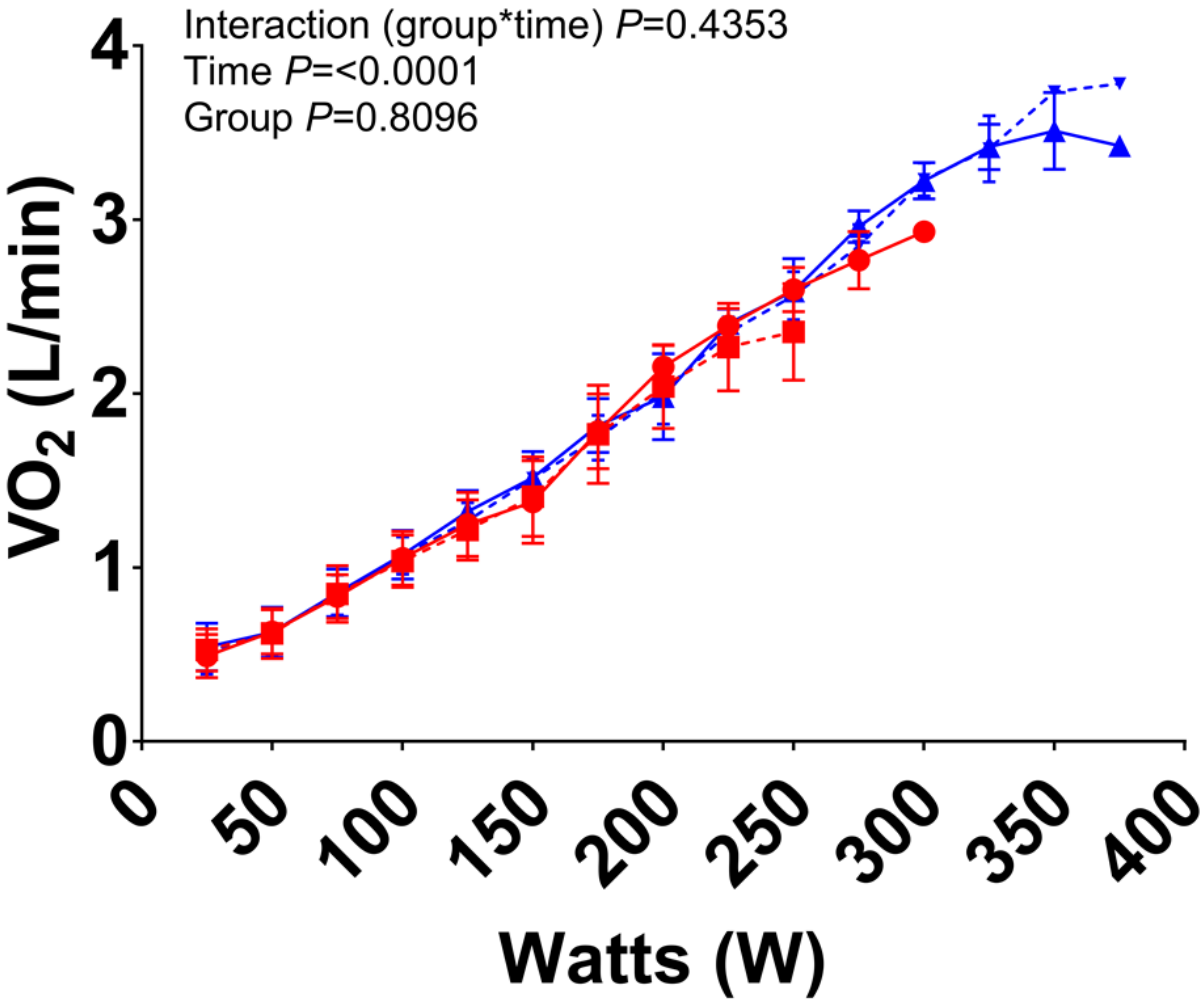


Figure 5-16 The $\dot{V}O2$ vs watts during a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

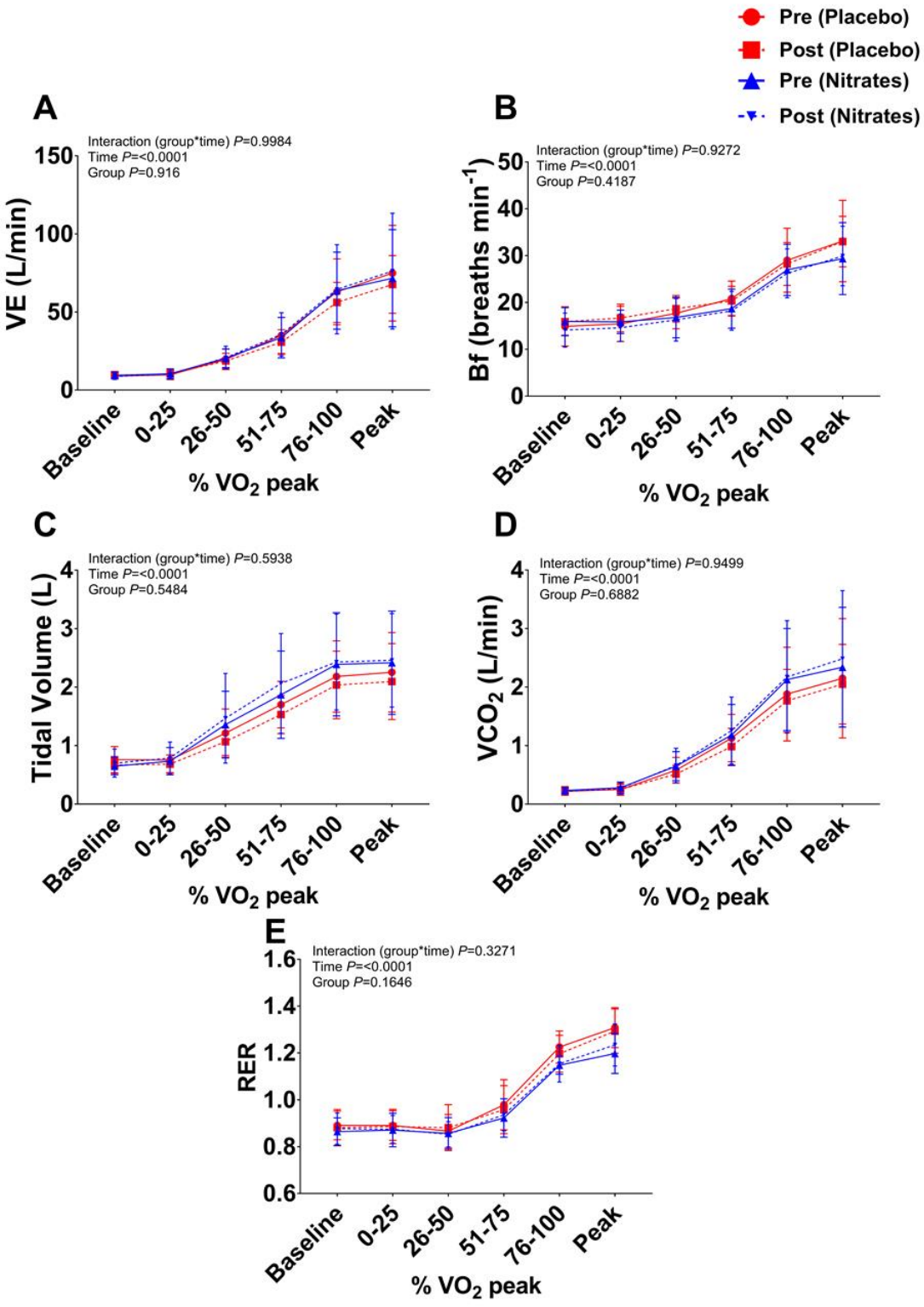


Figure 5-17 The effect of dietary nitrate or placebo on absolute respiratory measurements during cycle ergometer exercise testing ($\dot{V}O_2$ peak test).

A) minute ventilation (VE), B) breathing frequency and C) tidal volume D) volume of expired carbon dioxide (VCO_2) and E) the respiratory exchange ratio (RER) during a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

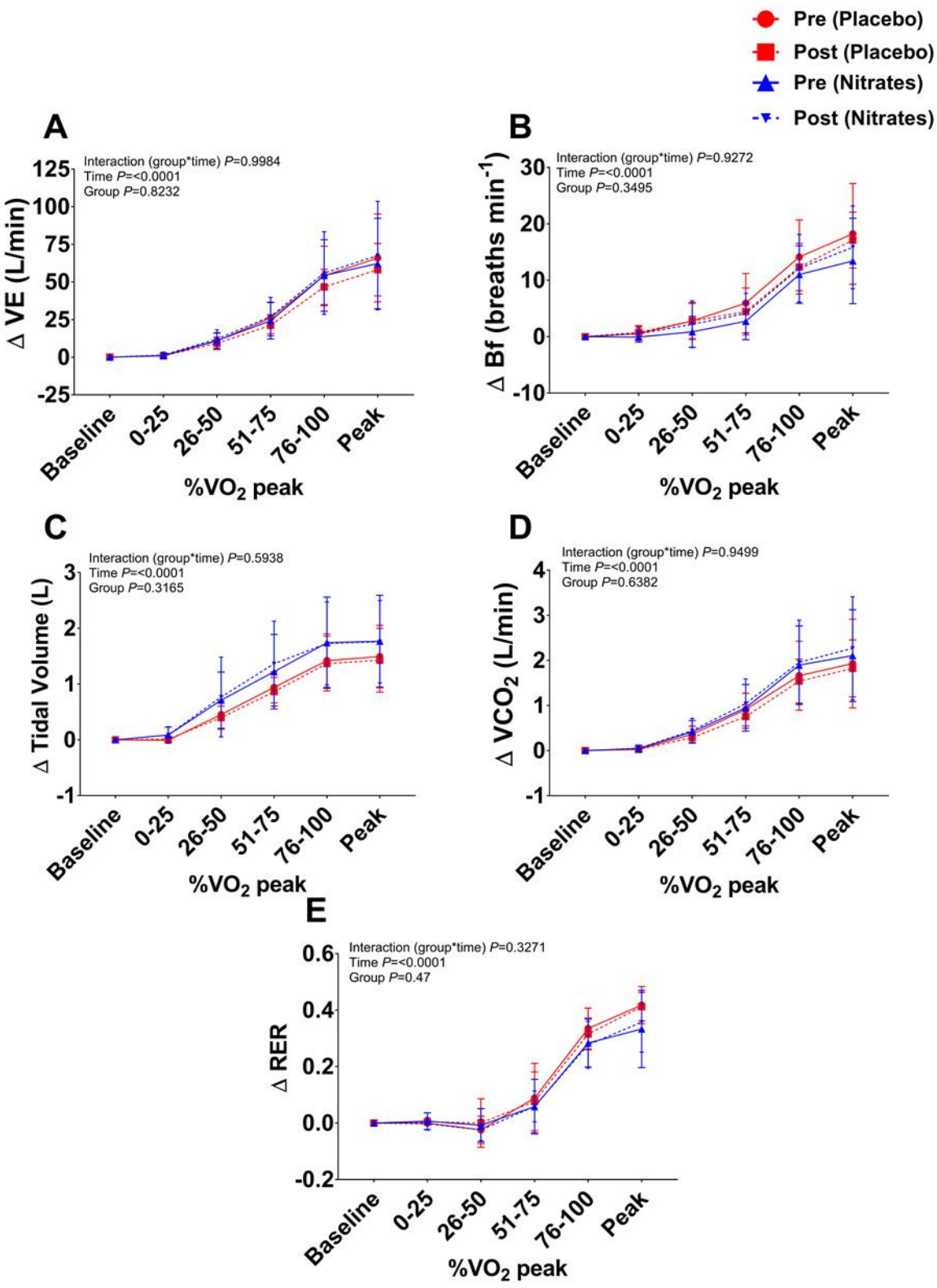


Figure 5-18 The effect of dietary nitrate or placebo on absolute change in respiratory measurements during cycle ergometer exercise testing ($\dot{V}O_2$ peak test).

A) minute ventilation (VE), B) breathing frequency and C) tidal volume D) volume of expired carbon dioxide (VCO_2) and E) the respiratory exchange ratio (RER) during a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

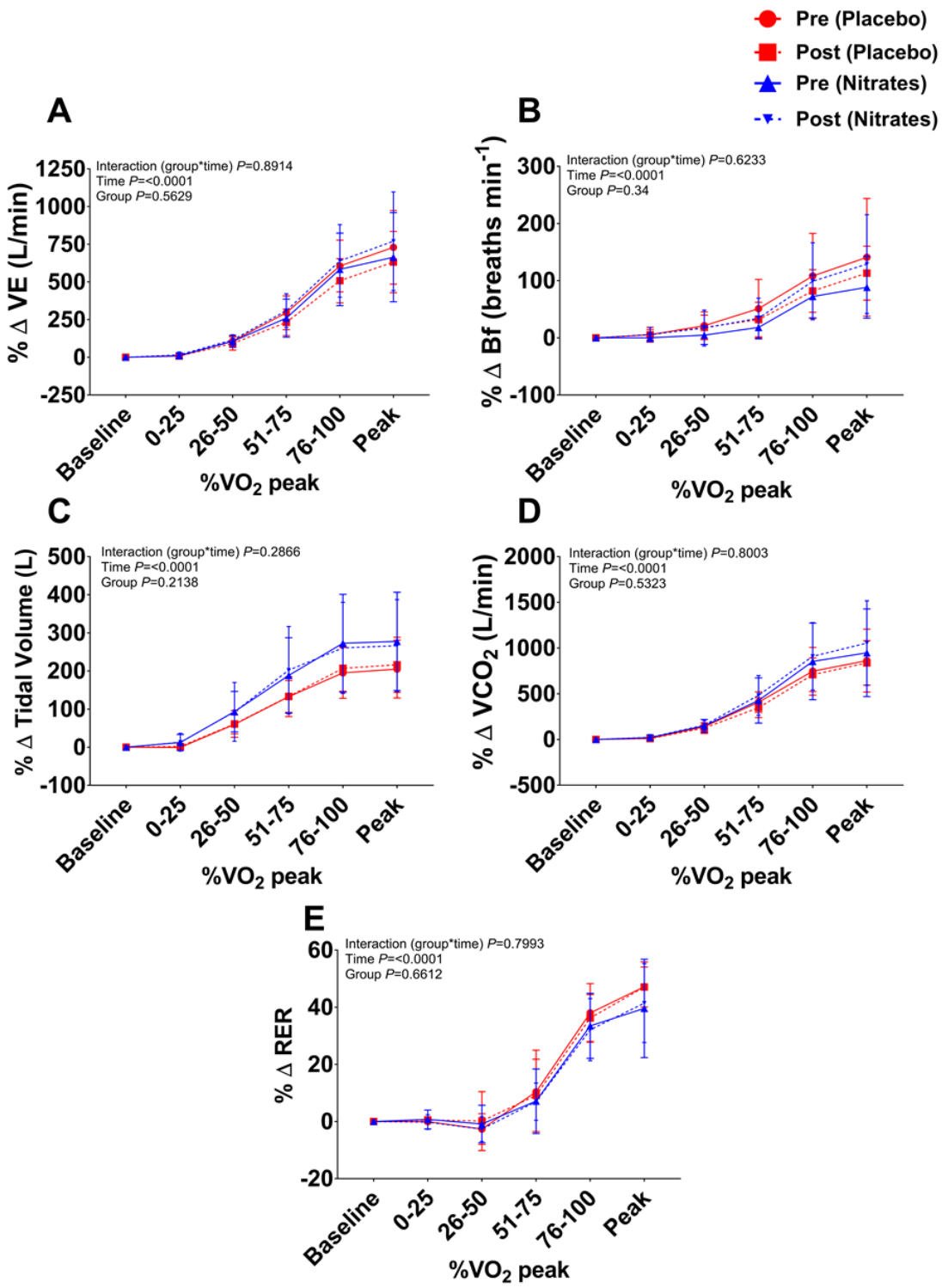


Figure 5-19 The effect of dietary nitrate or placebo on the % change in respiratory measurements during cycle ergometer exercise testing ($\dot{V}O_2$ peak test).

A) minute ventilation (VE), B) breathing frequency and C) tidal volume D) volume of expired carbon dioxide (VCO_2) and E) the respiratory exchange ratio (RER) during a peak cycle ergometer exercise test ($\dot{V}O_2$ peak test) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

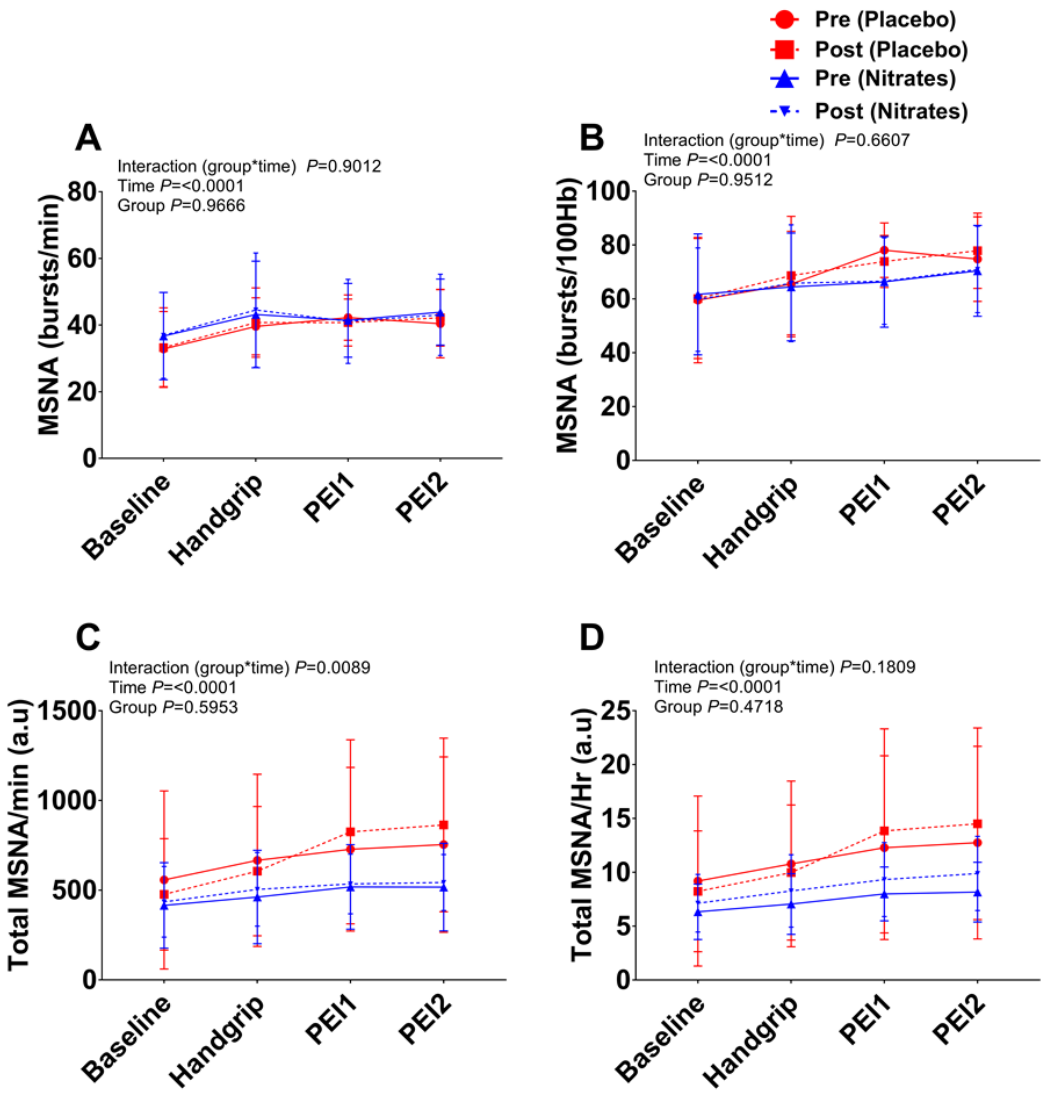


Figure 5-20 The effect of dietary nitrate or placebo on absolute muscle sympathetic nerve activity (MSNA) measurements during handgrip and metaboreflex testing.

A) MSNA burst frequency (bursts/min), B) MSNA burst incidence ((bursts/100Hb), C) MSNA area, D) total MSNA/min and E) total MSNA/Hr during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

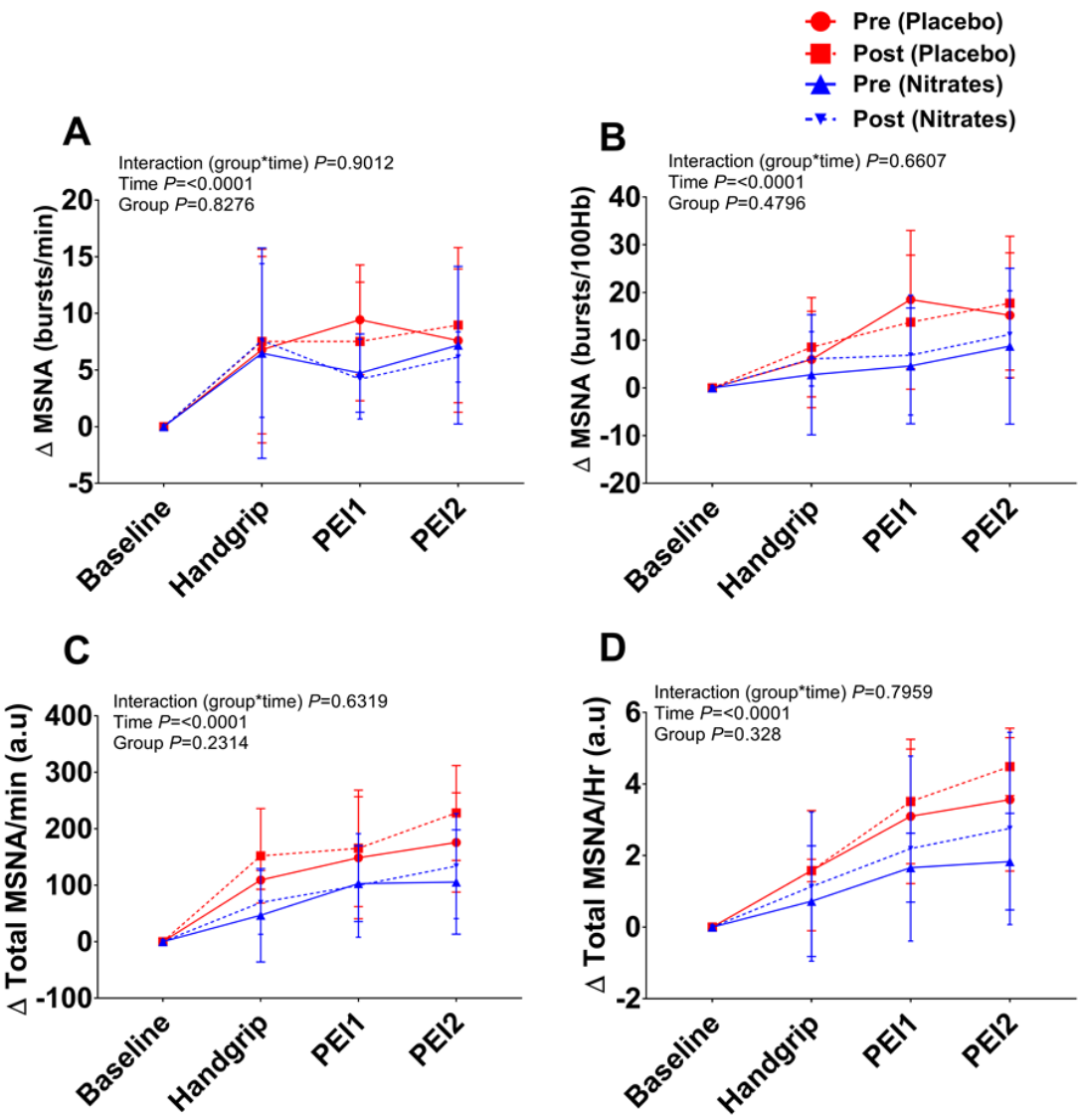


Figure 5-21 The effect of dietary nitrate or placebo on the change in muscle sympathetic nerve activity (MSNA) during handgrip and metaboreflex testing. A) MSNA burst frequency (bursts/min), B) MSNA burst incidence ((bursts/100Hb), C) MSNA area, D) total MSNA/min and E) total MSNA/Hr during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) from baseline pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

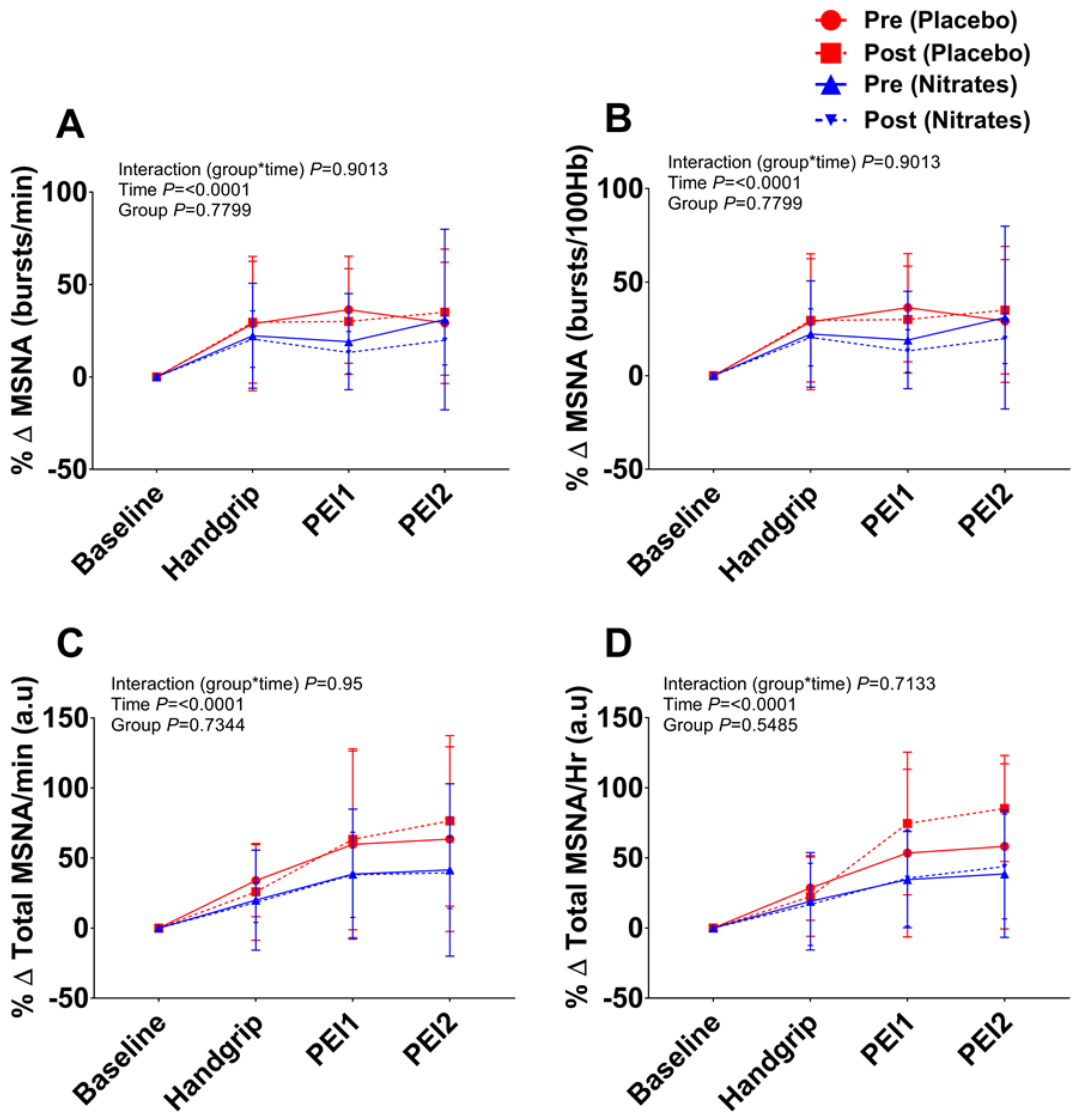


Figure 5-22 The effect of dietary nitrate or placebo on the % change in muscle sympathetic nerve activity (MSNA) during handgrip and metaboreflex testing. A) MSNA burst frequency (bursts/min), B) MSNA burst incidence (bursts/100Hb), C) MSNA area, D) total MSNA/min and E) total MSNA/Hr during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) from baseline pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

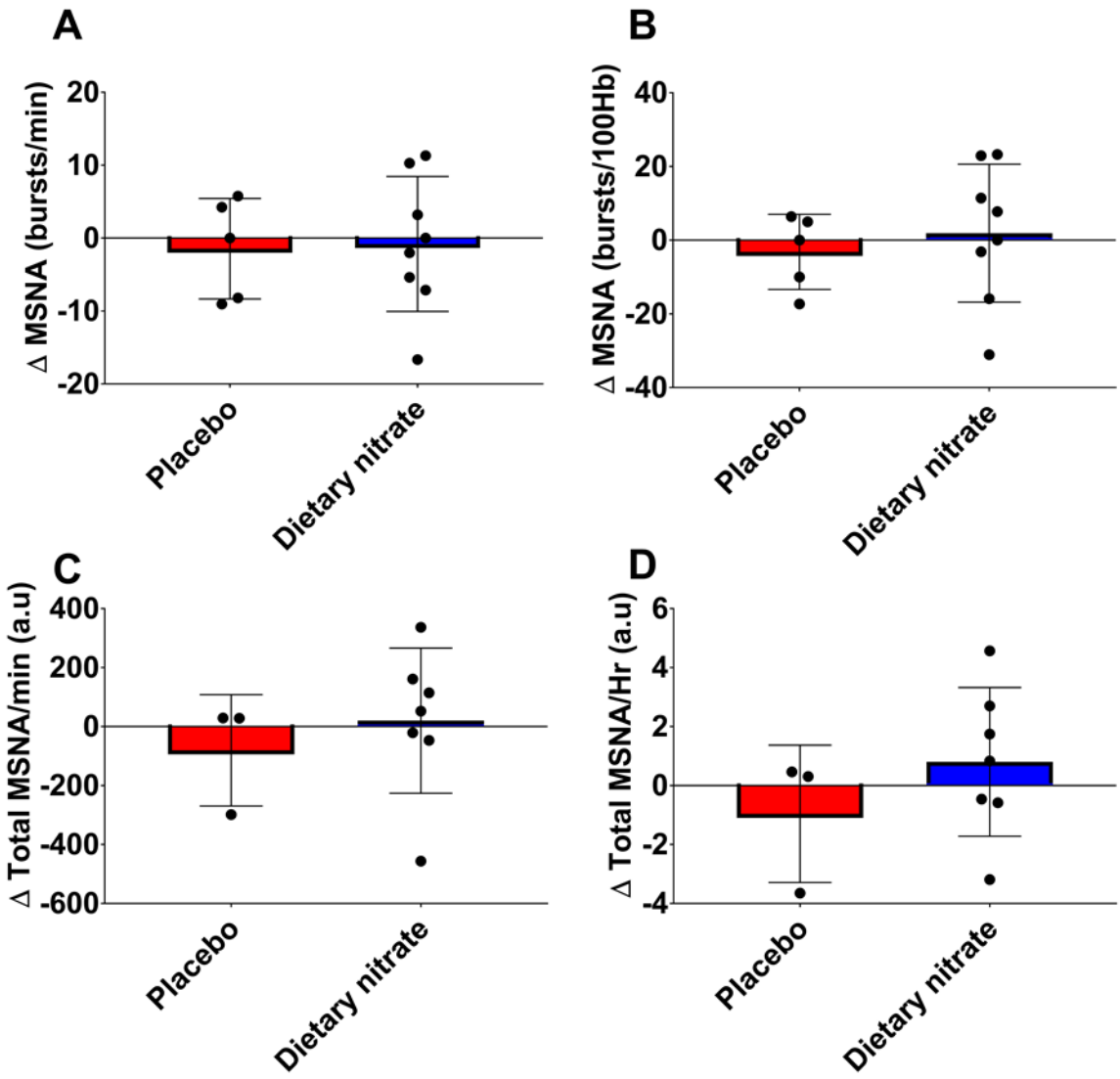


Figure 5-23 The effect of dietary nitrate or placebo on the change in muscle sympathetic nerve activity (MSNA) during 10 minutes of baseline.

A) muscle sympathetic nerve activity (MSNA) burst frequency (bursts/min), B) MSNA burst incidence (bursts/100Hb), C) total MSNA/min and D) total MSNA/Hr from pre to post intervention.

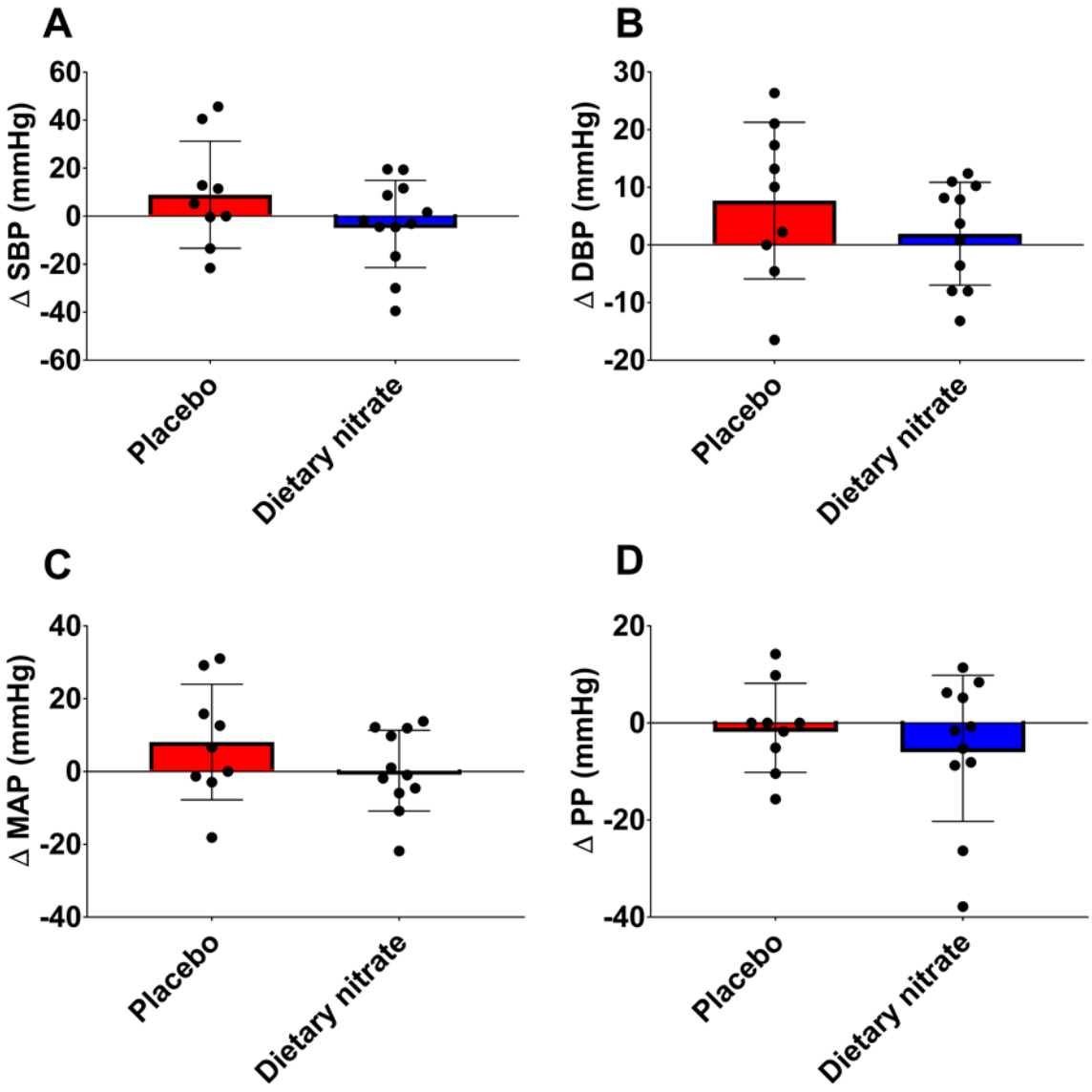


Figure 5-24 The effect of 4 weeks of dietary nitrate or placebo on the change in blood pressure during isometric handgrip testing at 40% MVC for 1 minute. A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) from pre to post intervention during isometric handgrip exercise.

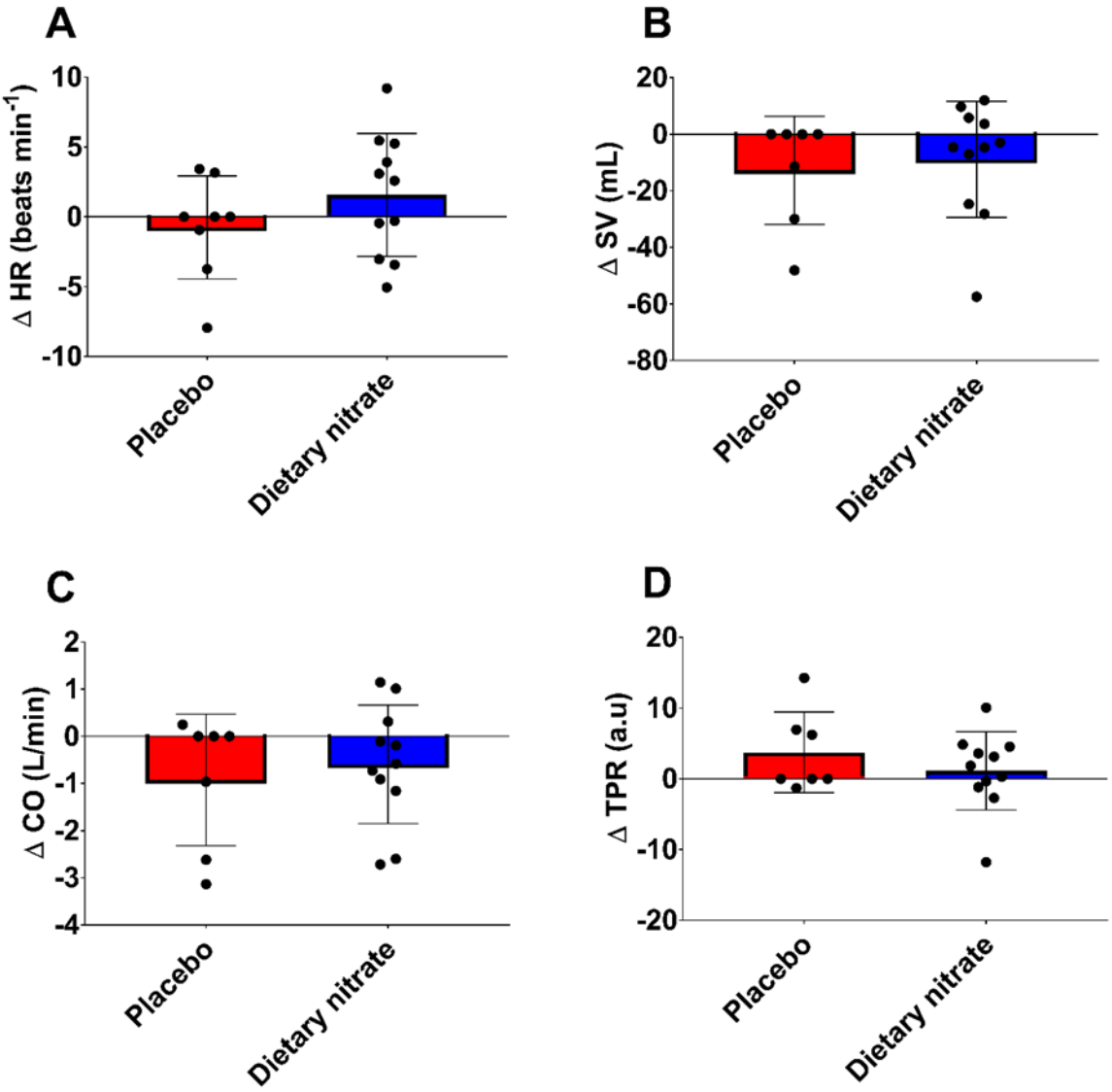


Figure 5-25 The effect of 4 weeks of dietary nitrate or placebo on the change in haemodynamics during isometric handgrip testing at 40% MVC for 1 minute. The effect of 4 weeks of dietary nitrate or placebo on the change in A) heart rate (HR), B) stroke volume (SV), C) cardiac output (CO) and D) total peripheral resistance (TPR) from pre to post intervention during isometric handgrip exercise.

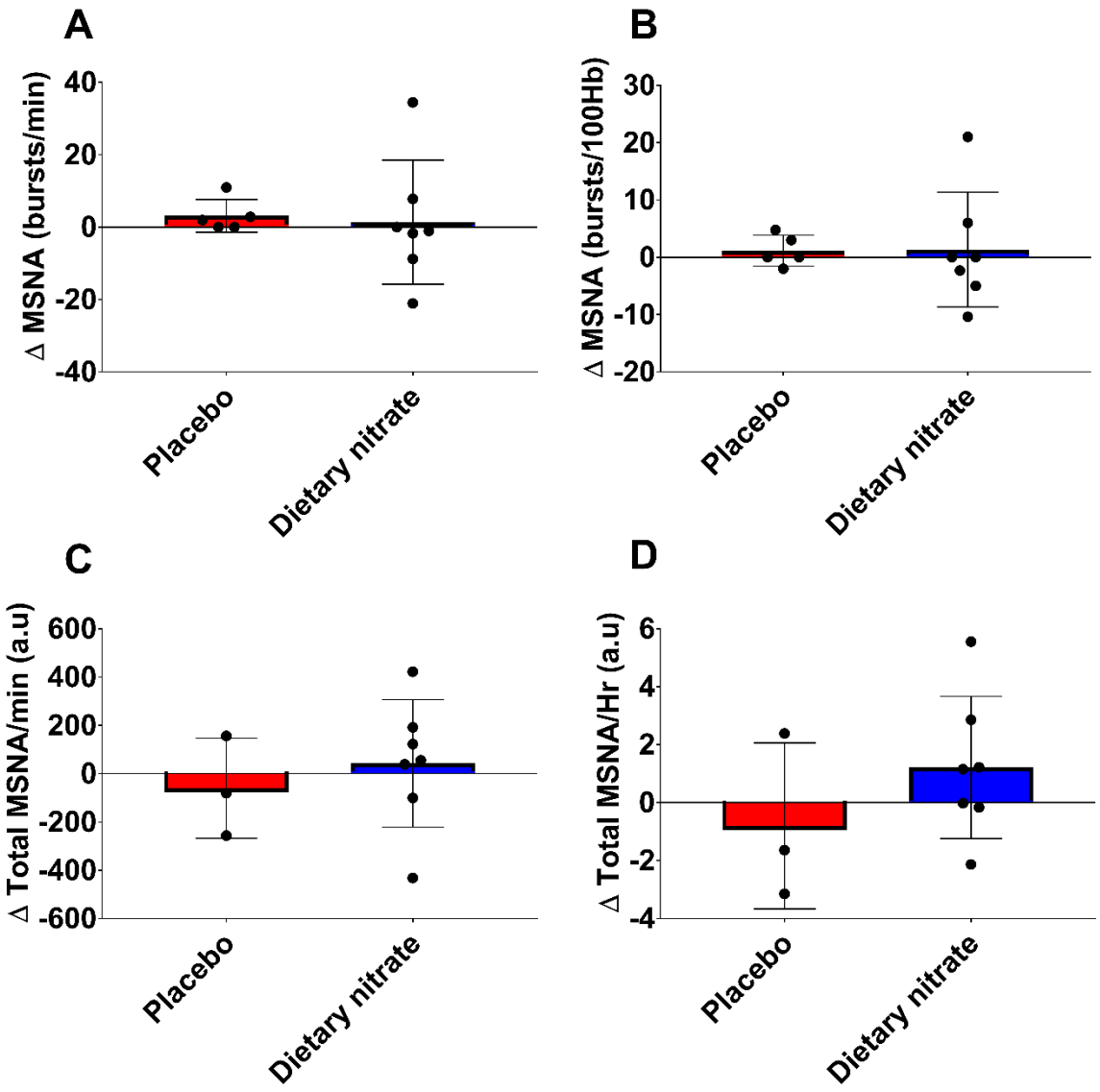


Figure 5-26 The effect of 4 weeks of dietary nitrate or placebo on the change in muscle sympathetic nerve activity (MSNA) during isometric handgrip testing. The effect of 4 weeks of dietary nitrate or placebo on the change in A) muscle sympathetic nerve activity (MSNA) burst frequency (bursts/min), B) MSNA burst incidence (bursts/100Hb), C) total MSNA/min and D) total MSNA/Hr from pre to post intervention.

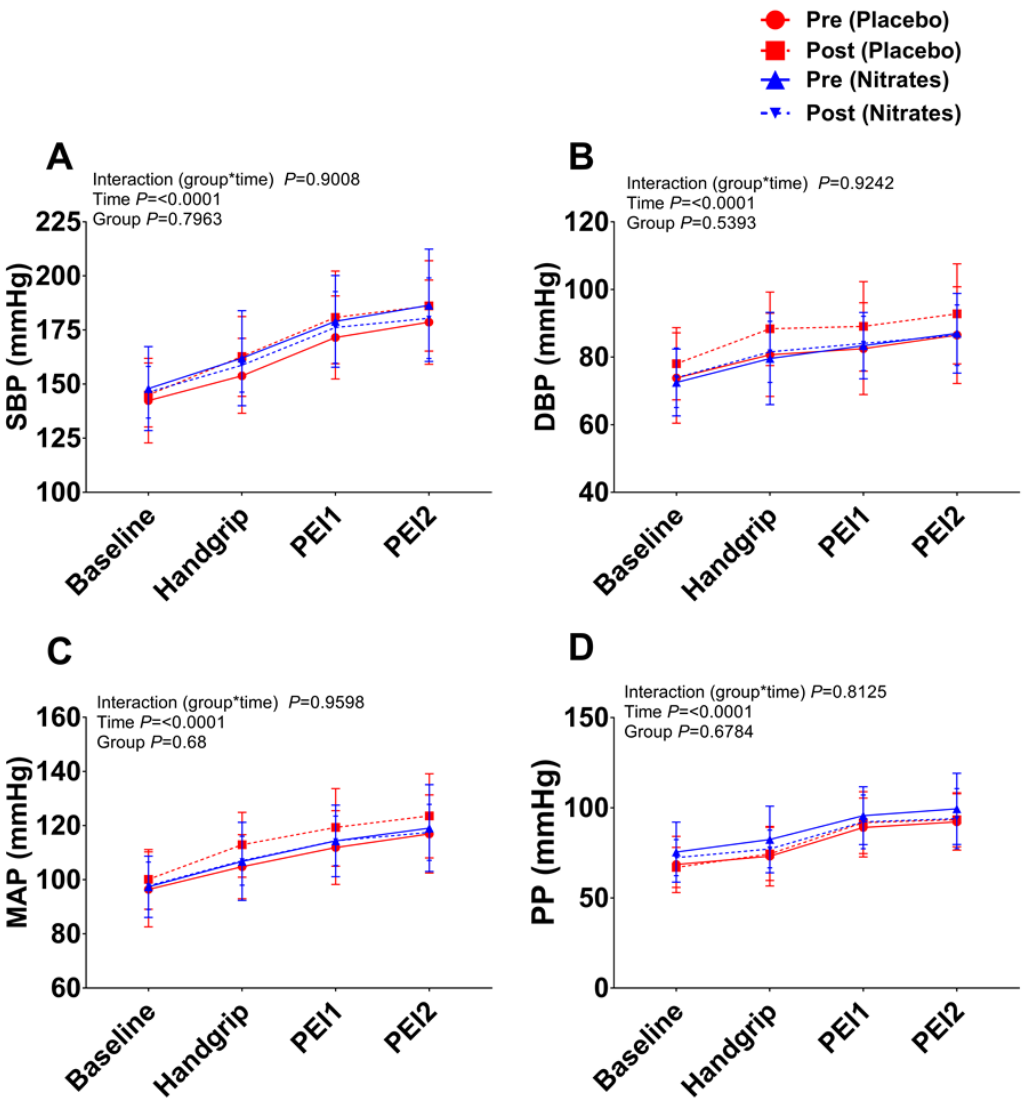


Figure 5-27 The effect of dietary nitrate or placebo on the absolute blood pressure during baseline, isometric handgrip exercise and metaboreflex testing. A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

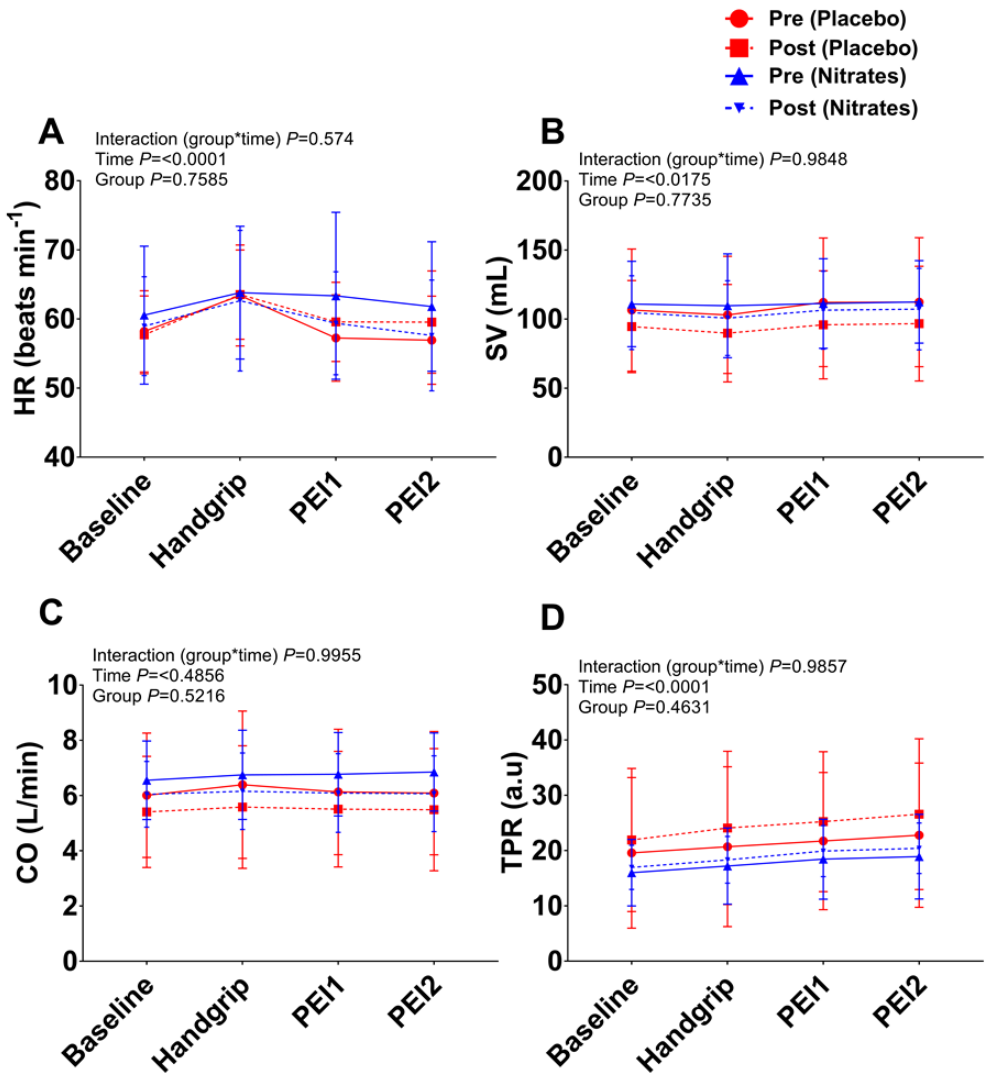


Figure 5-28 The effect of dietary nitrate or placebo on the absolute haemodynamics during baseline, isometric handgrip exercise and metaboreflex. A) heart rate (HR), B) stroke volume (SV), C) cardiac output (Q) and D) total peripheral resistance (TPR) during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

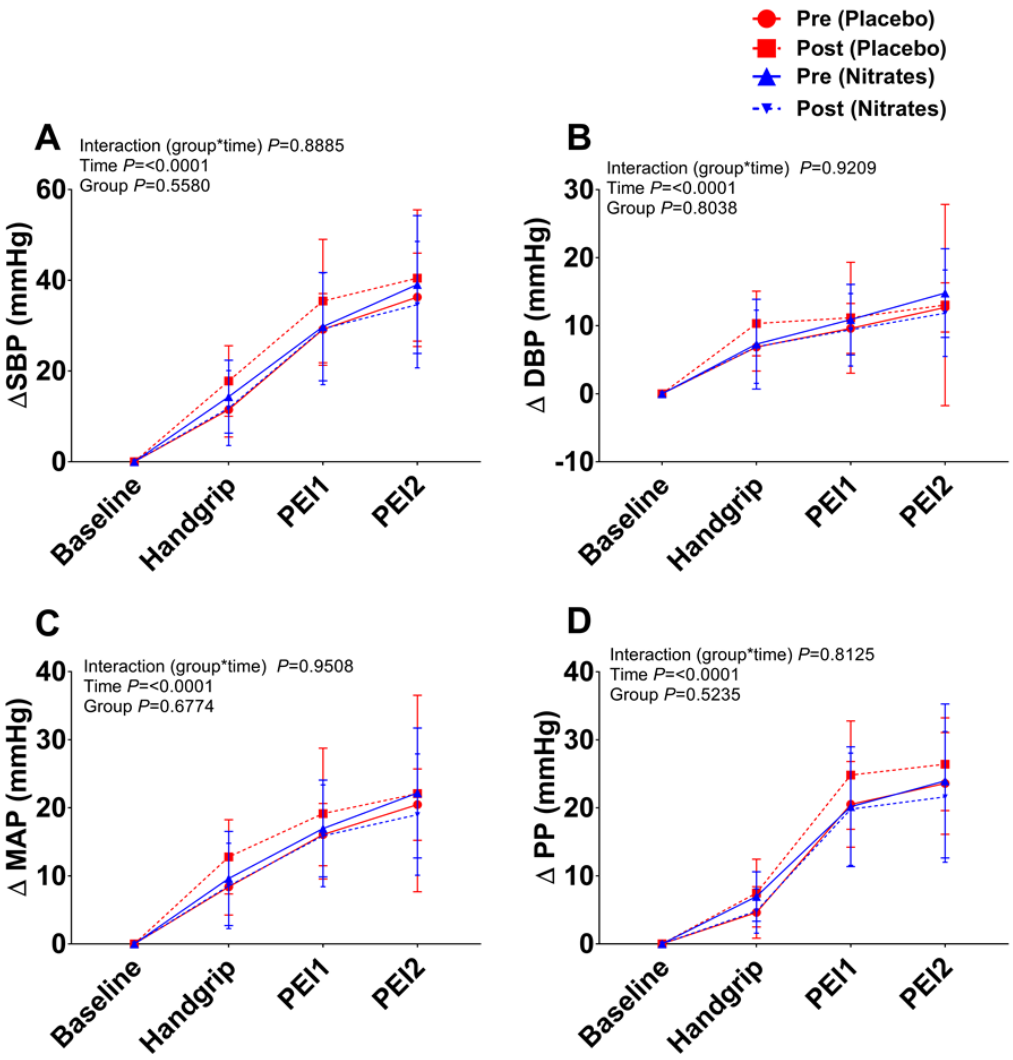


Figure 5-29 The effect of dietary nitrate or placebo on the change in absolute blood pressure during baseline, isometric handgrip exercise and metaboreflex. A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) from baseline during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

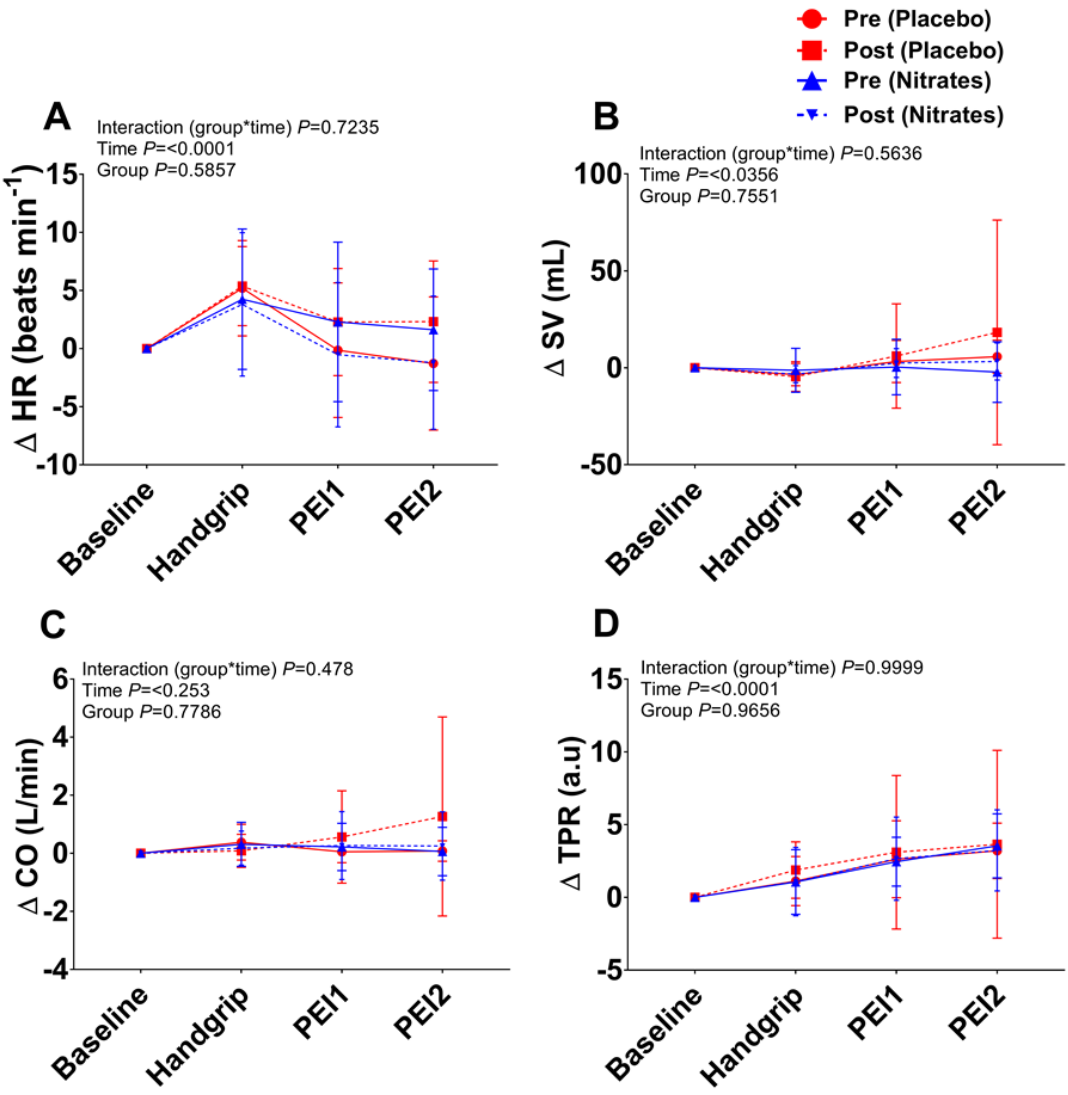


Figure 5-30 The effect of dietary nitrate or placebo on the absolute change in haemodynamics during baseline, isometric handgrip and metaboreflex testing. A) heart rate (HR), B) stroke volume (SV), C) cardiac output (Q) and D) total peripheral resistance (TPR) during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

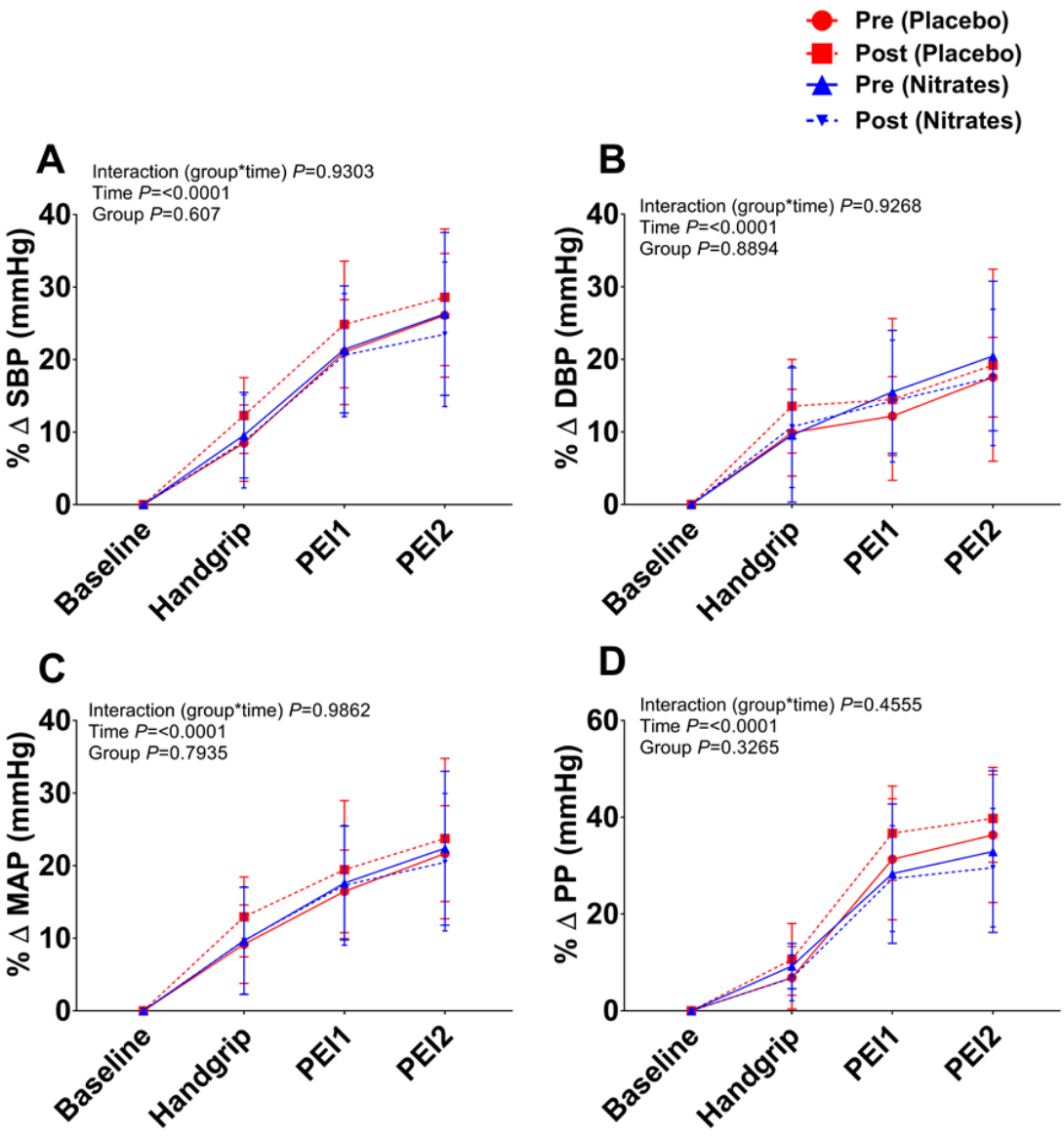


Figure 5-31 The effect of dietary nitrate or placebo on the % change in absolute blood pressure during baseline, isometric handgrip and metaboreflex testing. A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) from baseline during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

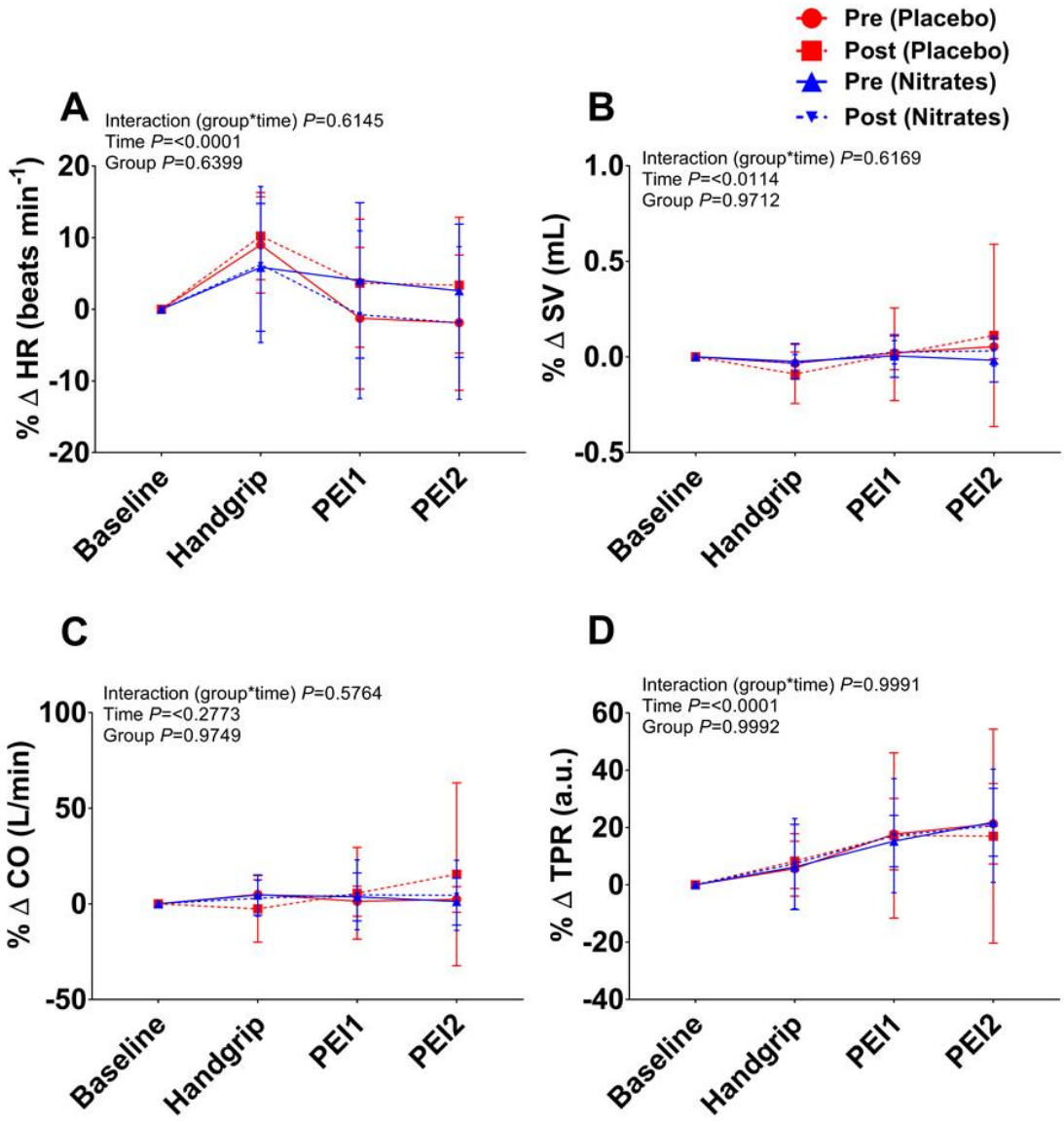


Figure 5-32 The effect of dietary nitrate or placebo on the % change in absolute haemodynamics during baseline, isometric handgrip and metaboreflex testing. A) heart rate (HR), B) stroke volume (SV), C) cardiac output (CO) and D) total peripheral resistance (TPR) during isometric handgrip exercise at 40% maximal voluntary contraction, the first minute of post-exercise ischemia (PEI1) and the second minute of post-exercise ischemia (PEI2) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

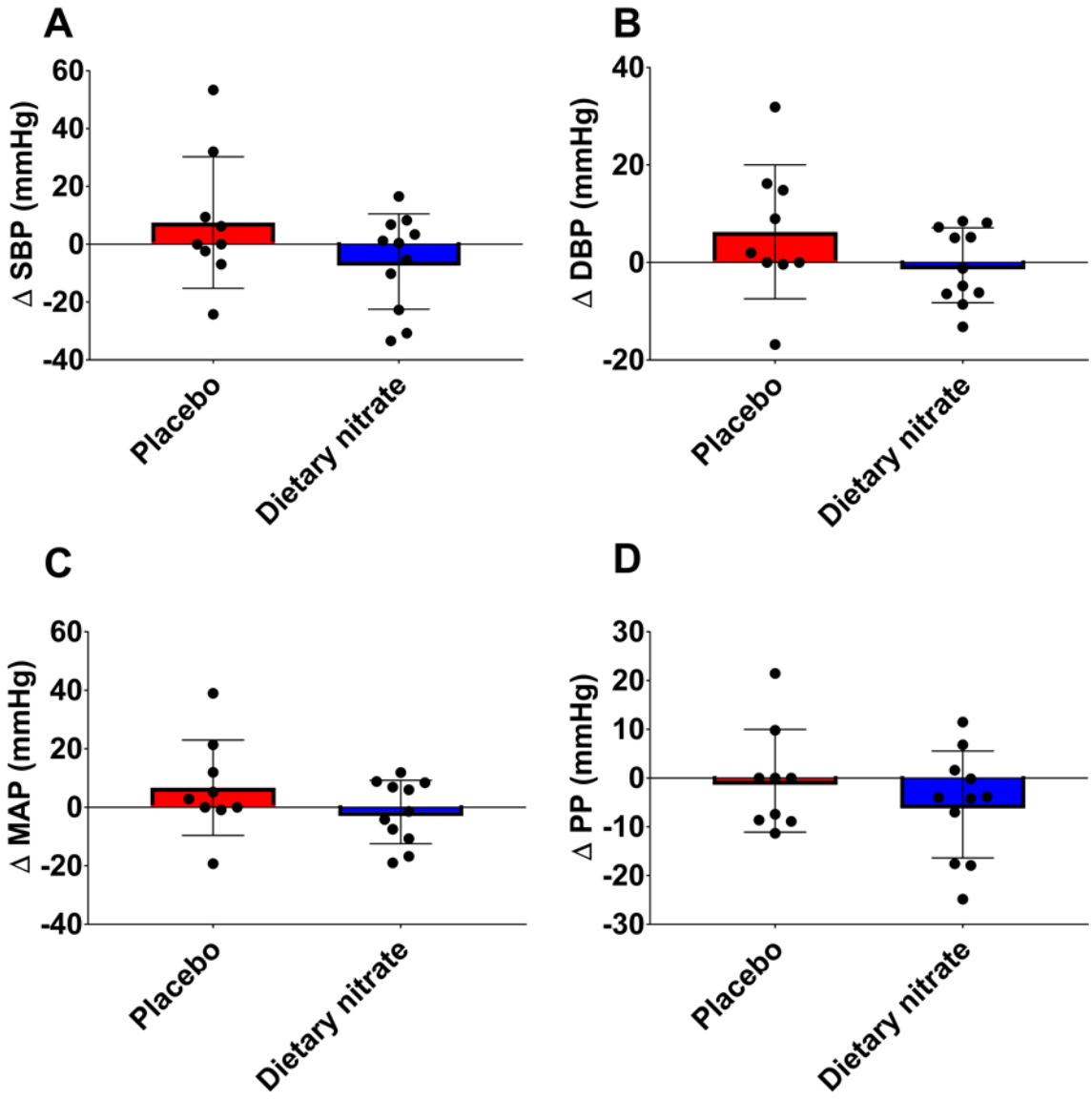


Figure 5-33 The effect of 4 weeks of dietary nitrate or placebo on the change in blood pressure during metaboreflex testing (post-exercise ischemia).

The change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) from pre to post intervention during the second minute of post-exercise ischemia (PEI2).

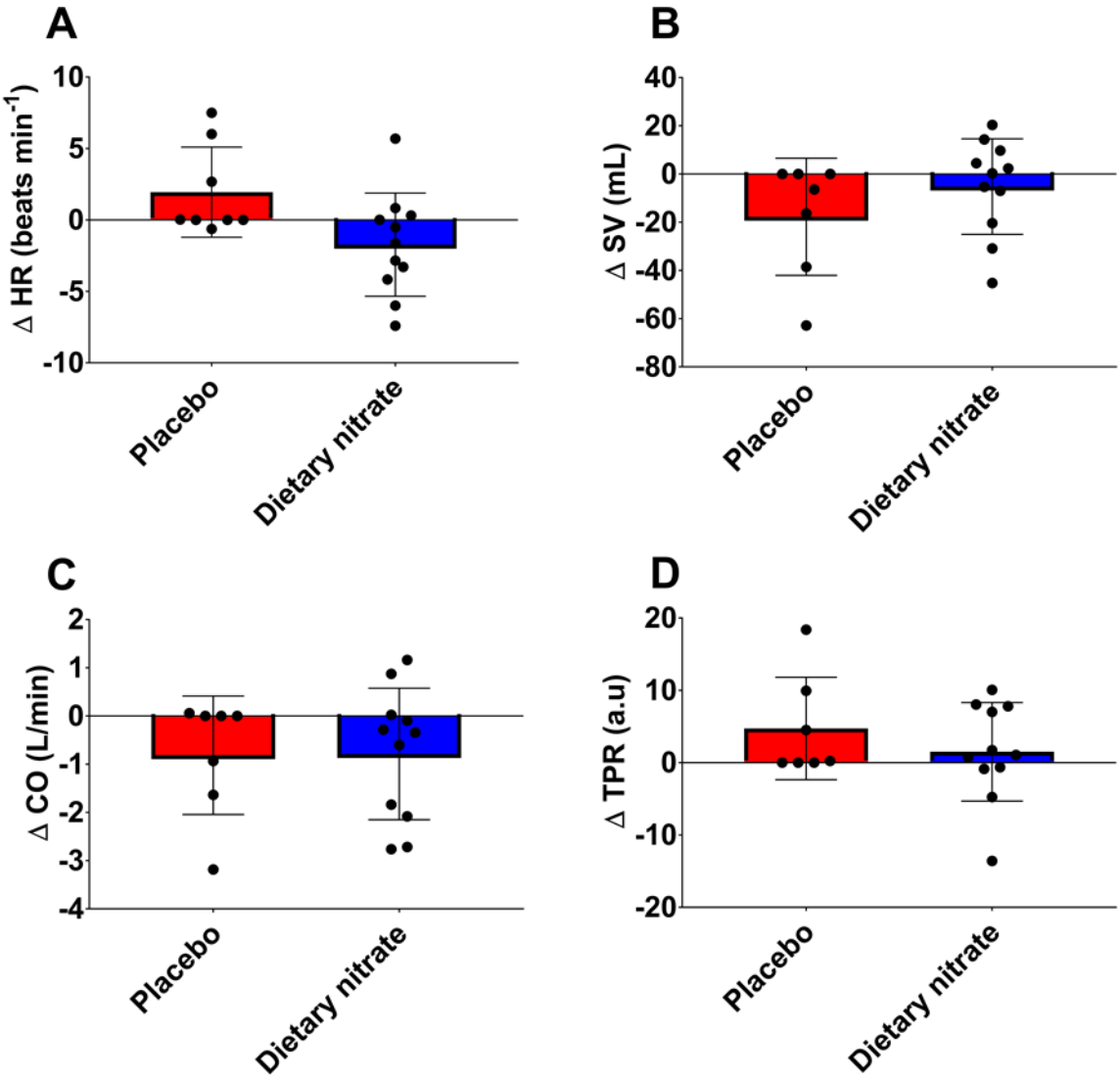


Figure 5-34 The effect of 4 weeks of dietary nitrate or placebo on the change in haemodynamics during metaboreflex testing (post-exercise ischemia).

The change in A) heart rate (HR), B) stroke volume (SV), C) cardiac output (CO) and D) total peripheral resistance (TPR) from pre to post intervention during the second minute of post-exercise ischemia (PEI2).

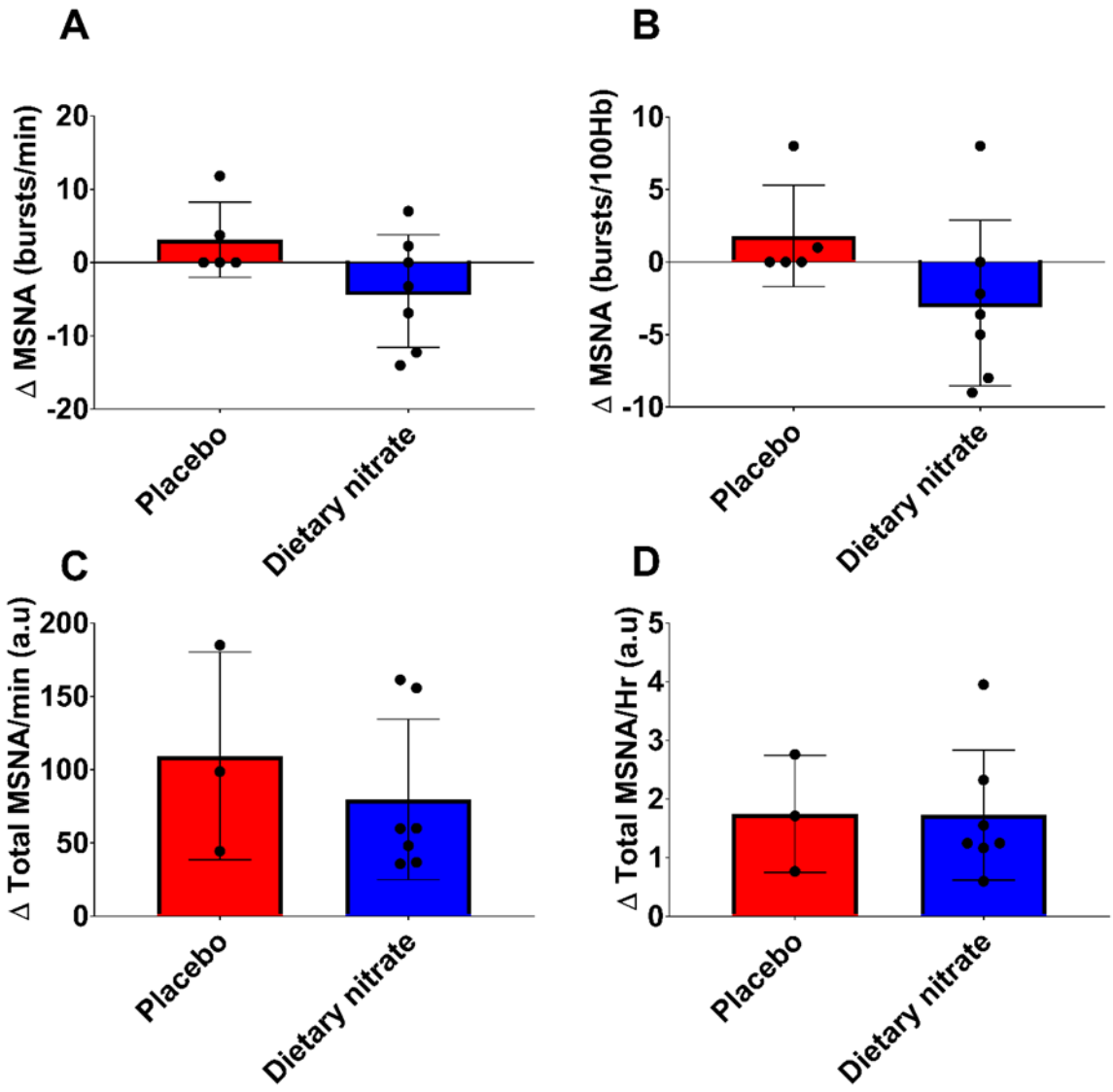


Figure 5-35 The effect of 4 weeks of dietary nitrate or placebo on the change in muscle sympathetic nerve activity (MSNA) during metaboreflex testing.

The change in A) MSNA burst frequency (bursts/min), B) MSNA burst incidence (bursts/100Hb), C) total MSNA/min and D) total MSNA/Hr from pre to post intervention during the second minute of post-exercise ischemia.

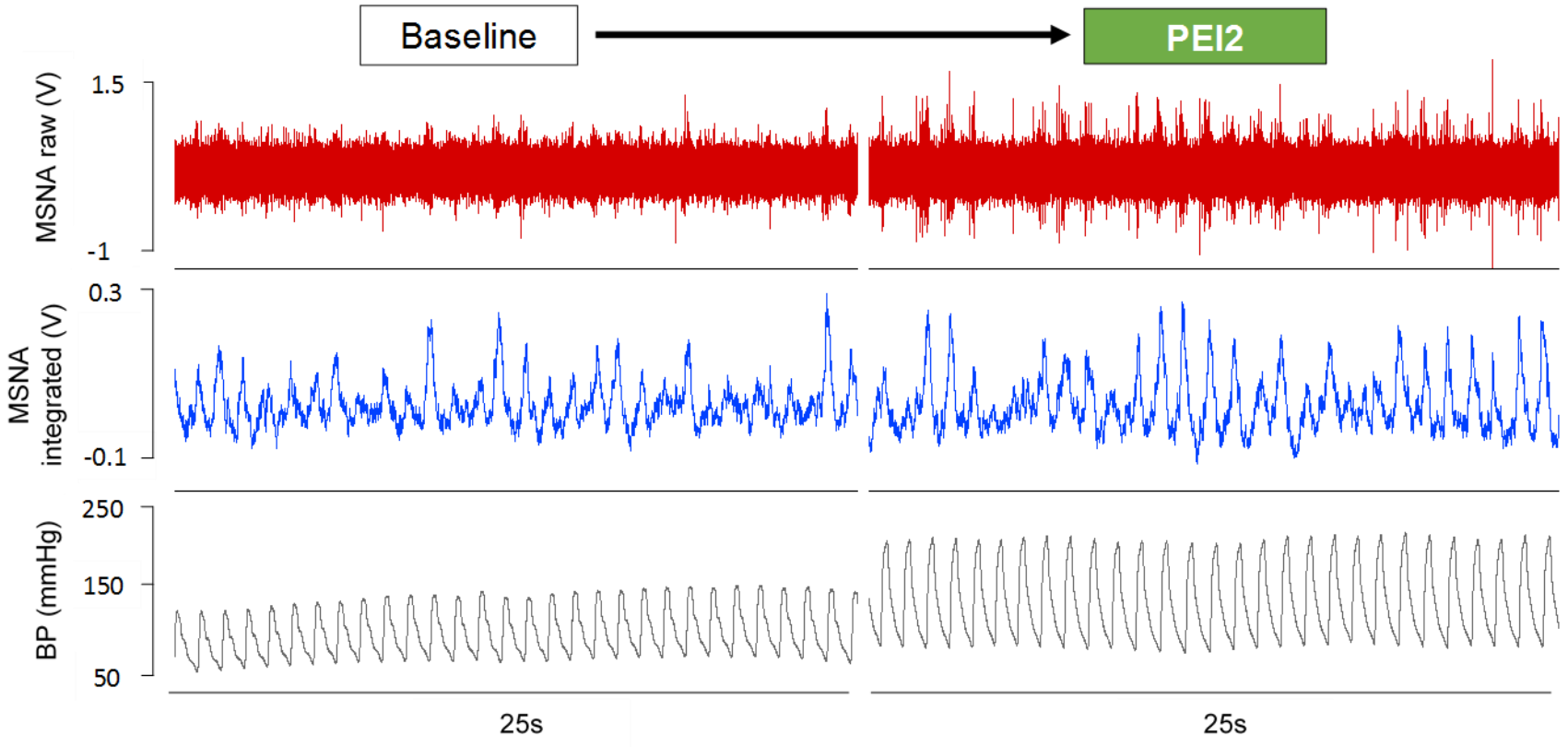


Figure 5-36 An example muscle sympathetic nerve activity (MSNA) trace (as measured by microneurography) at baseline and during post-exercise ischemia (PEI).

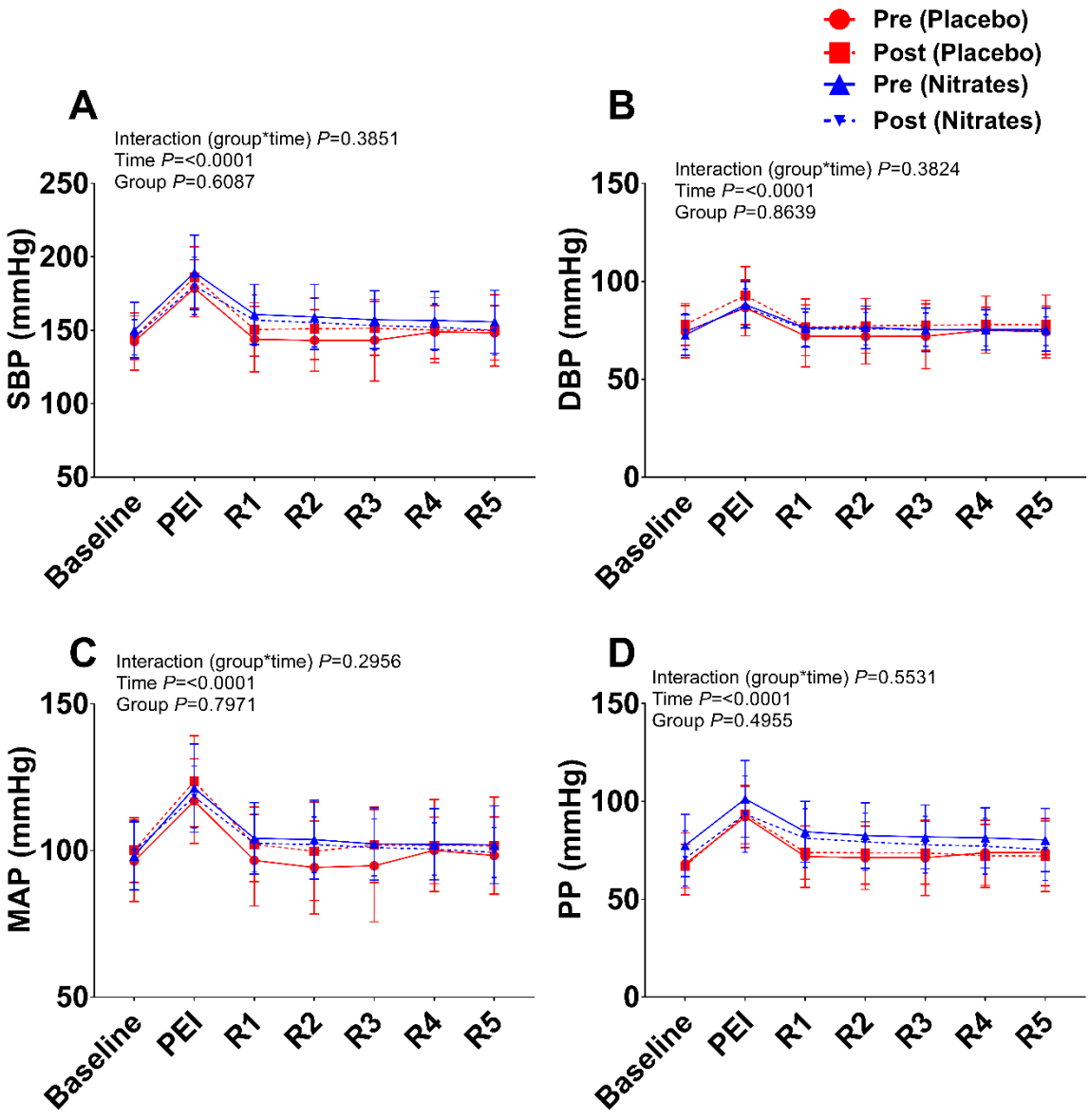


Figure 5-37 The effect of dietary nitrate or placebo on the absolute blood pressure during recovery from isometric handgrip and metaboreflex testing. The absolute A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

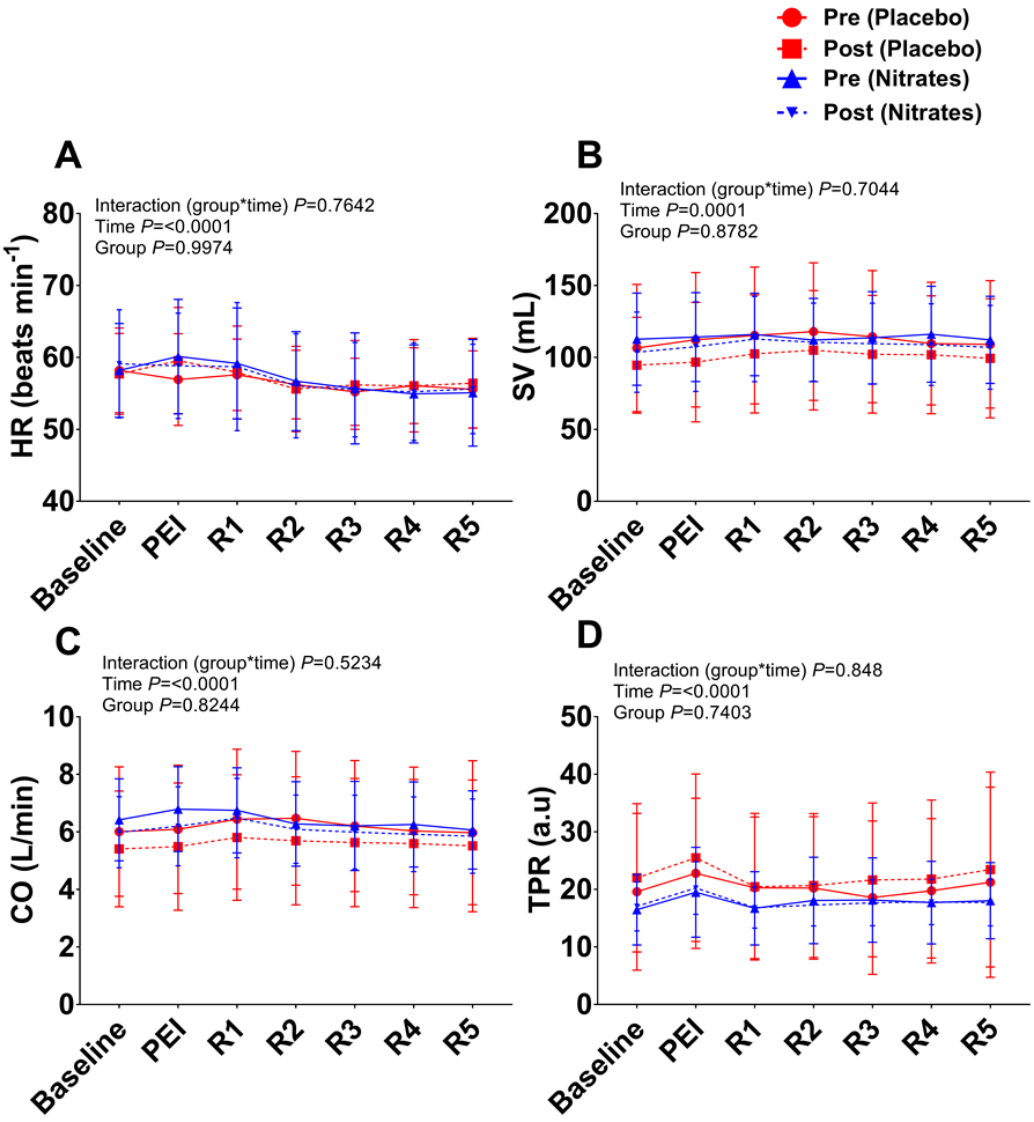


Figure 5-38 The effect of dietary nitrate or placebo on the absolute haemodynamic measurements during recovery from isometric handgrip and metaboreflex testing.

The absolute A) heart rate (HR), B) stroke volume (SV), C) cardiac output (CO) and D) total peripheral resistance (TPR) during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) from baseline pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

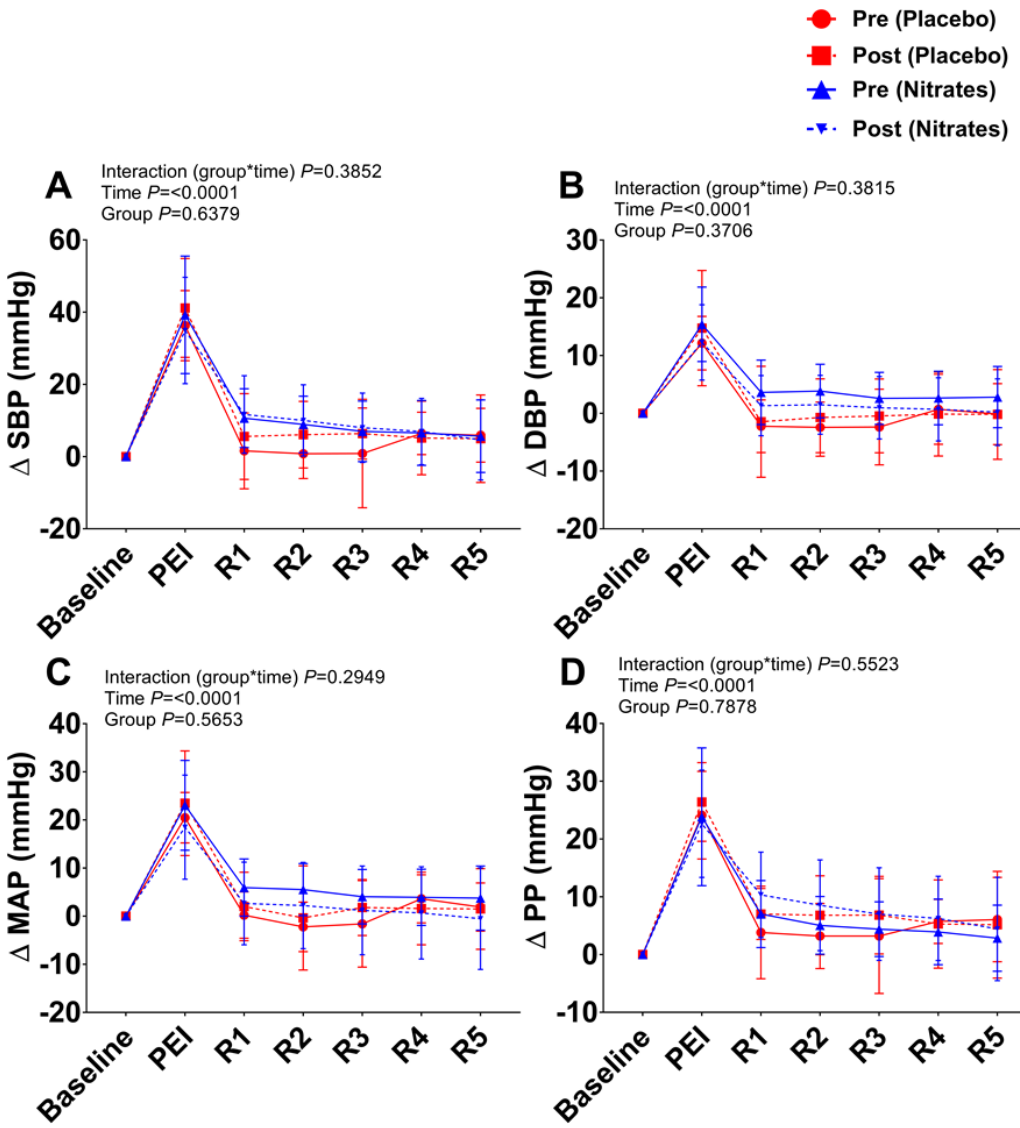


Figure 5-39 The effect of dietary nitrate or placebo on the absolute change in blood pressure during recovery from isometric handgrip and metaboreflex testing. The absolute change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) from baseline during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

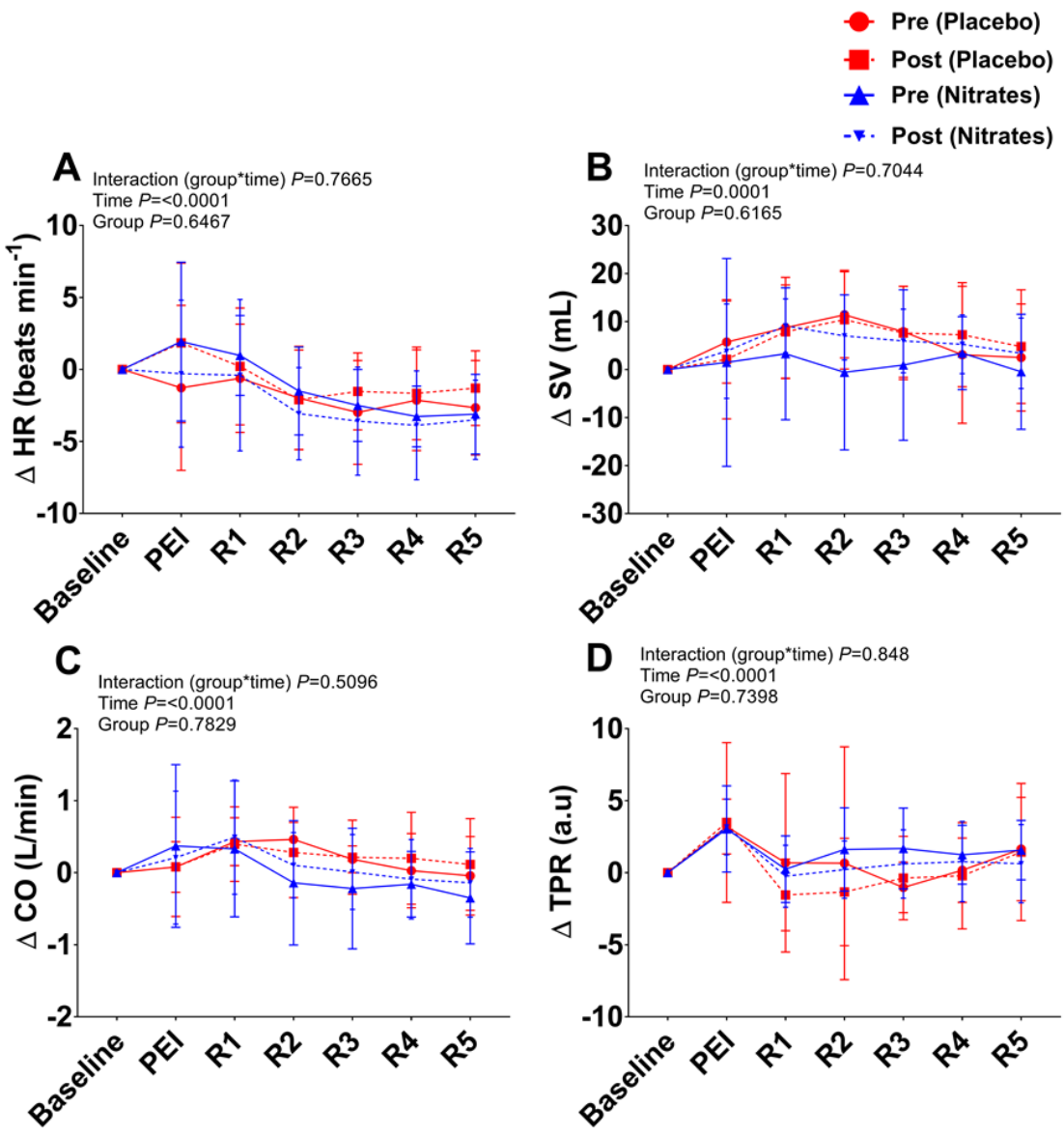


Figure 5-40 The effect of dietary nitrate or placebo on the absolute change in haemodynamics during recovery from isometric handgrip and metaboreflex. The absolute change in A) heart rate (HR), B) stroke volume (SV), C) cardiac output (CO) and D) total peripheral resistance (TPR) during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) from baseline pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

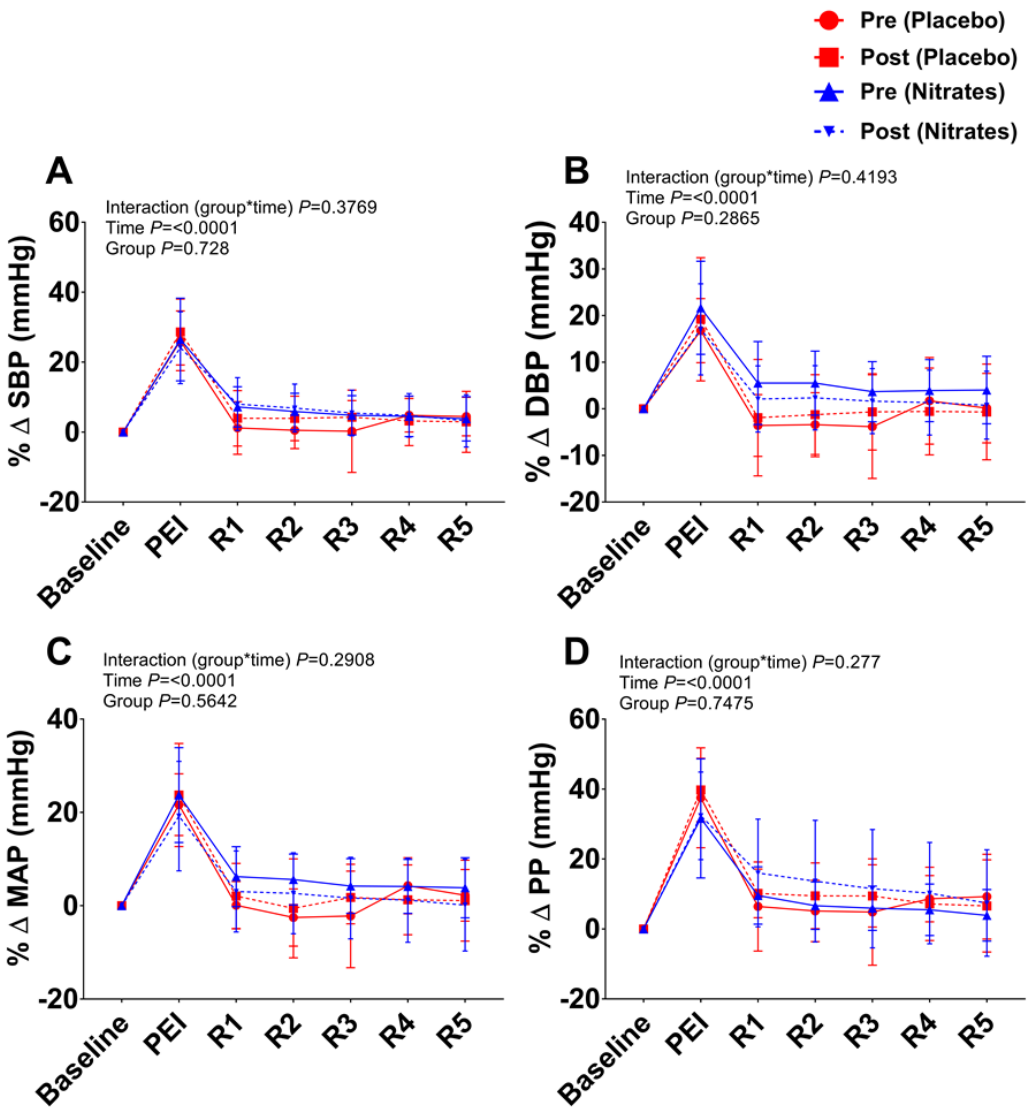


Figure 5-41 The effect of dietary nitrate or placebo on the % change in blood pressure during recovery from isometric handgrip and metaboreflex testing. The percentage (%) change in A) systolic blood pressure (SBP), B) diastolic blood pressure (DBP), C) mean arterial pressure (MAP) and D) pulse pressure (PP) from baseline during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

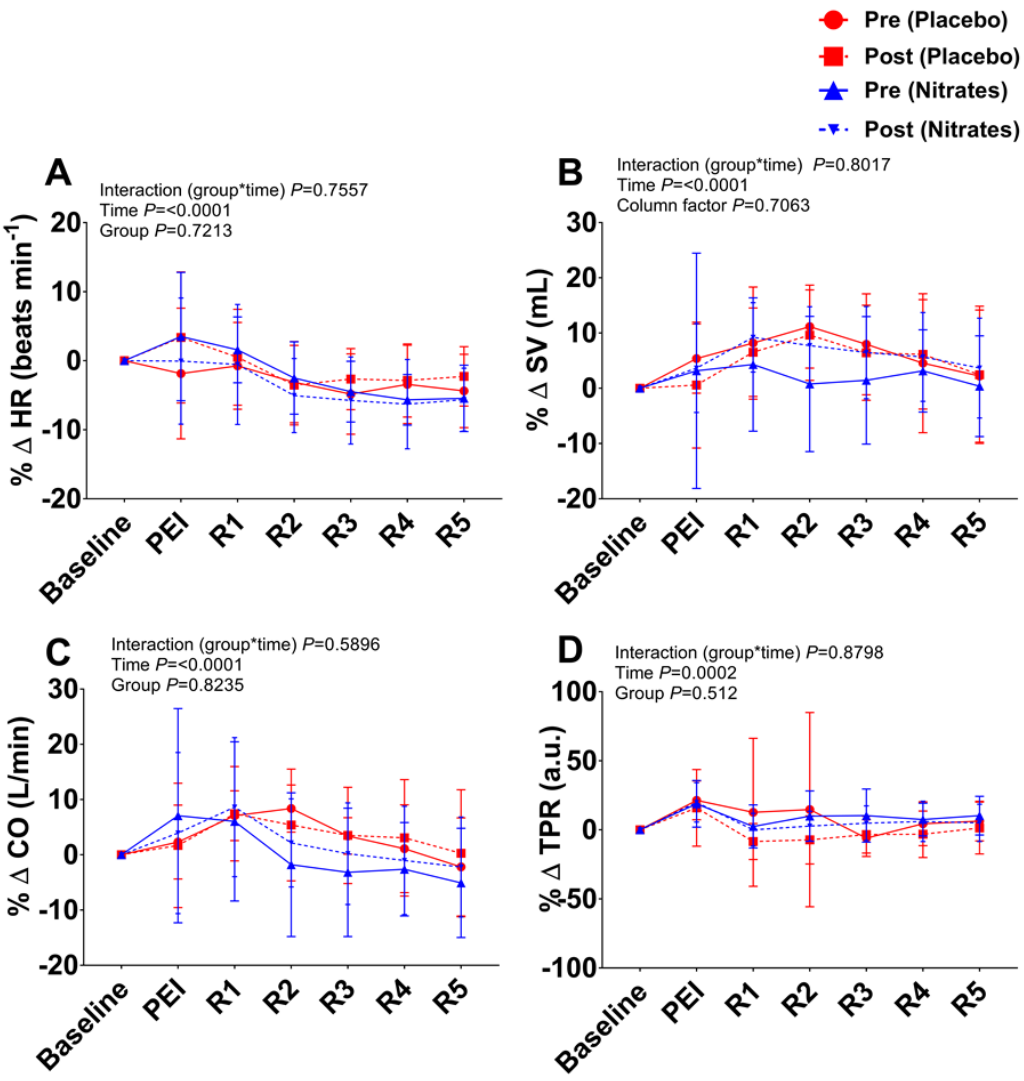


Figure 5-42 The effect of dietary nitrate or placebo on the % change in haemodynamics during recovery from isometric handgrip and metaboreflex. The percentage (%) change in A) heart rate (HR), B) stroke volume (SV), C) cardiac output (CO) and D) total peripheral resistance (TPR) during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) from baseline pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

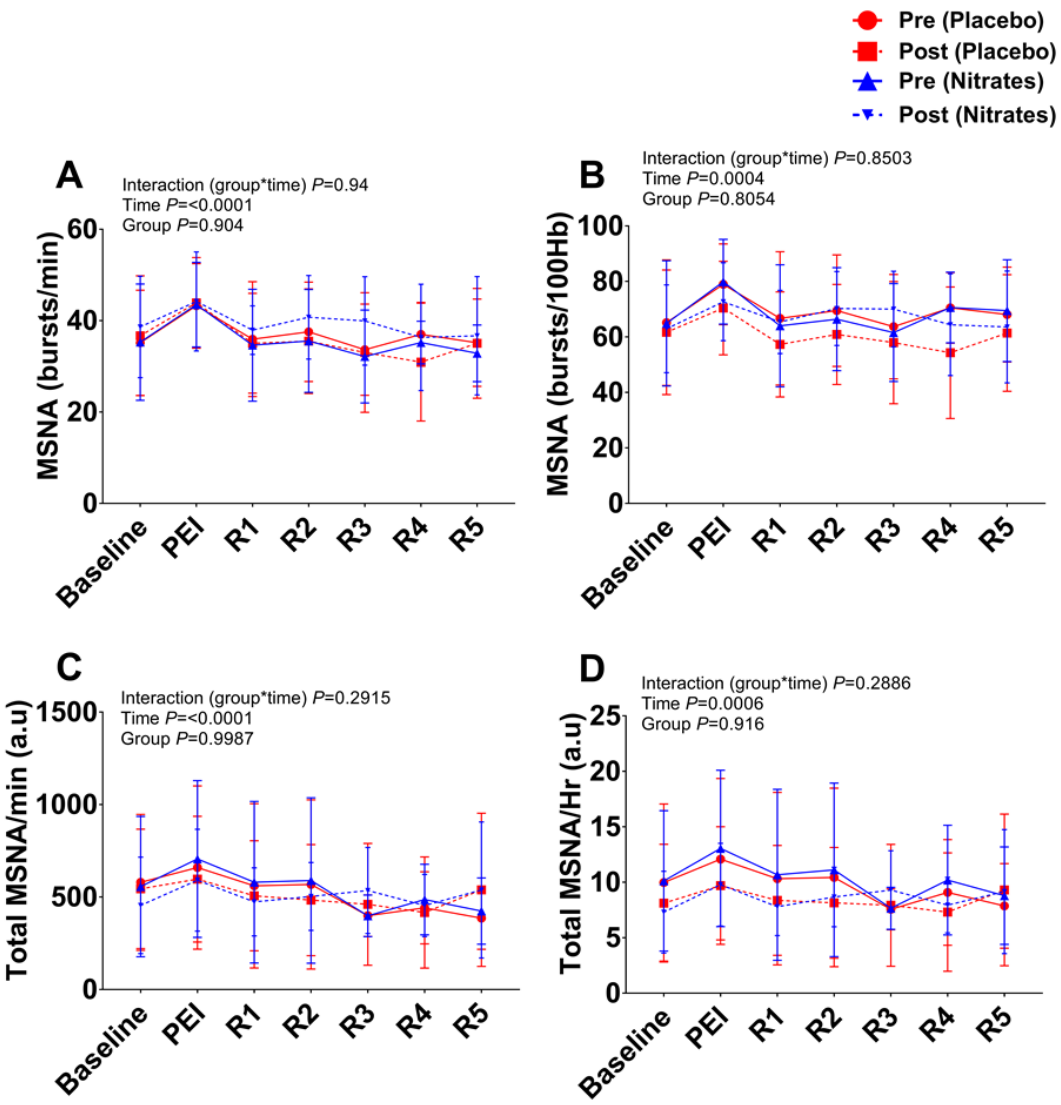


Figure 5-43 The effect of dietary nitrate or placebo on the absolute muscle sympathetic nerve activity (MSNA) during recovery from metaboreflex testing. The absolute A) muscle sympathetic nerve activity (MSNA) burst frequency (bursts/min), B) MSNA burst incidence (bursts/100Hb), C) total MSNA/min and D) total MSNA/Hr during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

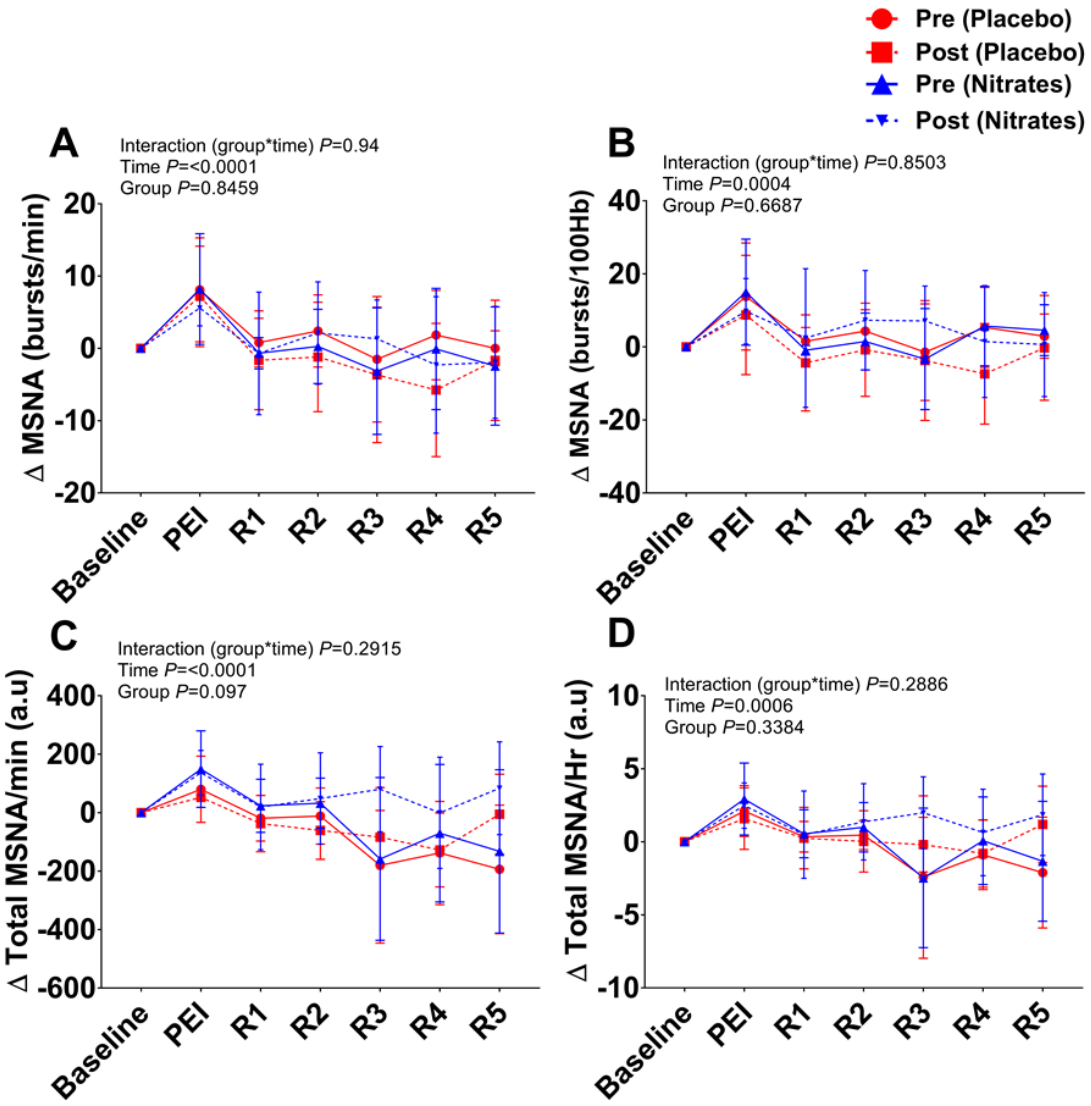


Figure 5-44 The effect of dietary nitrate or placebo on absolute change in muscle sympathetic nerve activity (MSNA) during recovery from metaboreflex testing. The absolute change in A) muscle sympathetic nerve activity (MSNA) burst frequency (bursts/min), B) MSNA burst incidence (bursts/100Hb), C) total MSNA/min and D) total MSNA/Hr during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

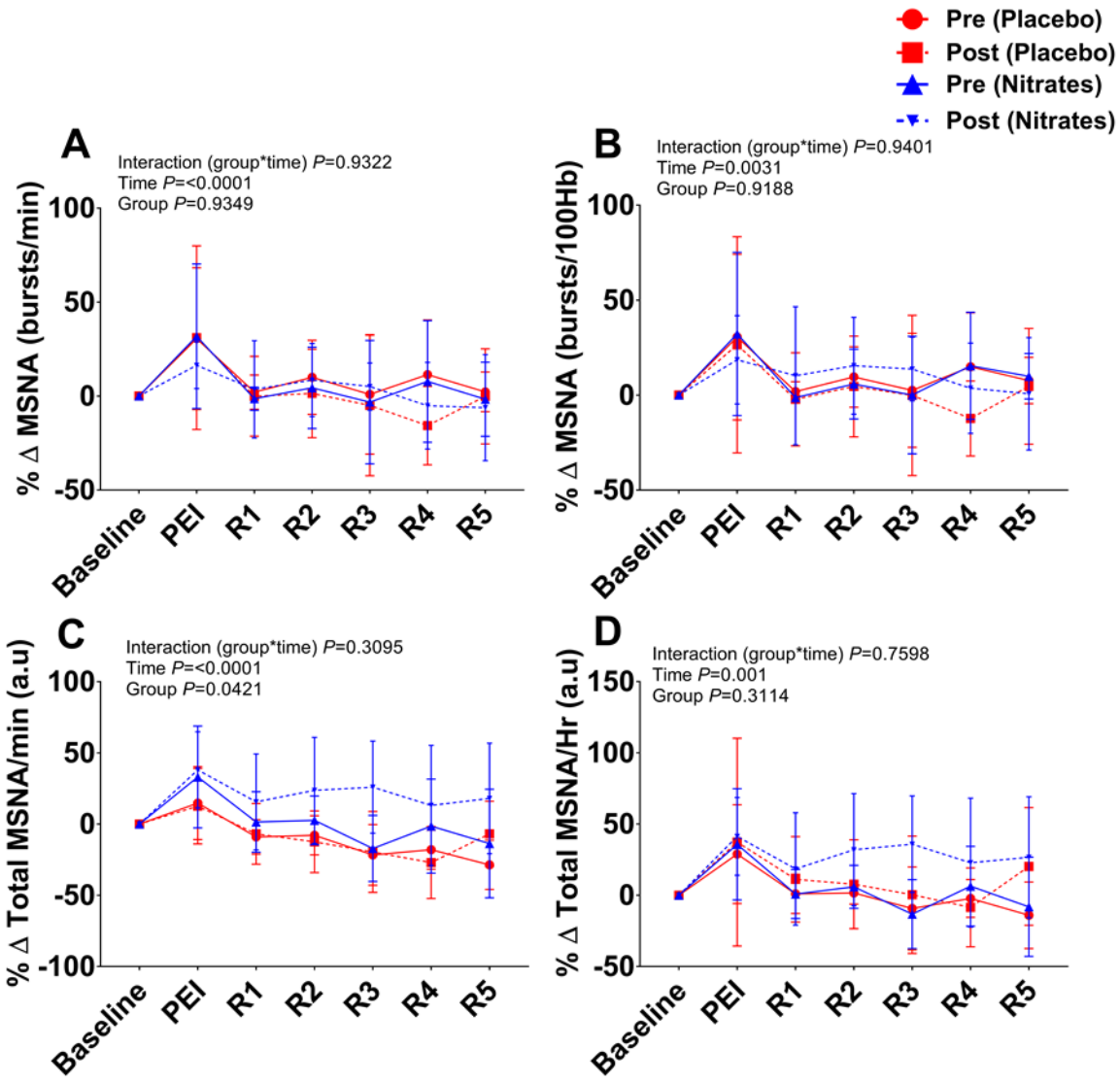


Figure 5-45 The effect of dietary nitrate or placebo on the absolute muscle sympathetic nerve activity (MSNA) during recovery from metaboreflex testing. The percentage change in A) muscle sympathetic nerve activity (MSNA) burst frequency (bursts/min), B) MSNA burst incidence (bursts/100Hb), C) total MSNA/min and D) total MSNA/Hr during the second minute of post-exercise ischemia (PEI) and the 5 one-minute epochs of recovery (R1, R2, R3, R4 and R5) pre and post 4 weeks of dietary nitrates or nitrate depleted placebo. Pre-placebo (red solid line), post-placebo (red dashed line), pre-dietary nitrates (blue solid line) and post-dietary nitrates (blue dashed line).

Chapter 6 General Discussion

Prior to the research in this thesis, it was unclear whether adequately controlling blood pressure (BP) with antihypertensive medications normalises the BP response to exercise and reduces metaboreflex hyperreflexia in human hypertension. The main aim of this thesis was to assess whether adequate control of BP with anti-hypertensive medication normalises the exaggerated pressor response to exercise that is associated with untreated hypertension. The overall hypothesis was that anti-hypertensive medication will have no effect on the cardiovascular response to exercise in hypertension and that treatments that reduce metaboreflex hyperreflexia in hypertension will prove more beneficial.

6.1 Summary of key findings

6.1.1 Antihypertensive medications fail to normalise the blood pressure response to peak exercise and metaboreceptor isolation

In support of the first hypothesis of this thesis, it was found that first-line antihypertensive treatment (as per NICE guidelines) fail to control BP during exercise. More specifically, treated controlled, uncontrolled and untreated hypertensives had an exaggerated BP response to maximal exercise testing on a cycle ergometer ($\dot{V}O_2$ peak test) when compared to normotensive controls. In addition, the BP response to metaboreflex isolation using post-exercise ischemia (PEI) was increased in treated controlled, uncontrolled and untreated hypertensives when compared to normotensives.

6.1.2 Aortic blood pressure is related to the exaggerated blood pressure response to exercise in hypertension

In contrast to the hypothesis tested in Chapter 4, only aortic BP was elevated in treated controlled and uncontrolled hypertensives. Pulse wave velocity was only elevated in untreated hypertensives and was similar among treated controlled, uncontrolled and normotensive individuals. A forced entry multiple linear regression was used to make a model to assess the contribution of aortic pulse pressure (PP), pulse wave velocity, age, daytime ambulatory peripheral SBP and the change in peripheral SBP during metaboreflex isolation on the change in SBP during submaximal (51-75% of $\dot{V}O_2$ peak) and peak exercise. During submaximal (51-75% of $\dot{V}O_2$ peak) exercise the only predictor of the SBP was the SBP response to metaboreflex isolation. At peak exercise ($\dot{V}O_2$ peak) only the SBP response to metaboreflex isolation and the resting aortic PP were predictive of peak SBP.

6.1.3 Dietary NO_3^- for 4 weeks fails to lower the systolic blood pressure response to peak exercise and metaboreflex isolation

In contrast to the tertiary main hypothesis in this thesis, chronic dietary NO_3^- intake had no effect on the peak SBP to incremental exercise and metaboreflex isolation in treated controlled hypertension. This was despite an elevation in both plasma NO_3^- and NO_2^-. However, group size was very small and the levels of plasma NO_3^- and NO_2^- following the active intervention were highly variable.

6.2 Treating an exaggerated blood pressure response to exercise

Drawing the results from this thesis together, alternative medications or therapies are needed that decrease the BP response to exercise in patients with treated controlled hypertension. The data from this thesis has highlighted that the metaboreflex and aortic BP are important contributors to an exaggerated BP response to exercise.

Elevated aortic BP may indicate reduced aortic compliance. An elevated aortic stiffness and aortic characteristic impedance in hypertension would lead to exaggerated brachial artery BP because a reduced capacity to expand during exercise-induced increases in stroke volume would cause A) an increased forward wave from the left ventricle and B) the left ventricle to have to work harder to eject blood. Treatments that target elevated aortic stiffness would likely reduce the brachial artery BP during exercise and also reduce the work of the left ventricle. The current consensus is that angiotensin converting enzyme inhibitors (ACEi) are most effective at lowering aortic pressures at rest (Boutouyrie et al., 2011). However, 44% of treated uncontrolled hypertension and 88% of treated controlled hypertension were taking an ACEi in the study in Chapter 4. Further research is needed to establish the most effective method for reducing aortic stiffness in patients with hypertension.

It is well established that an exaggerated BP response to exercise is an integrated response, involving several feedback mechanisms (mechanoreflex and metaboreflex), feed-forward mechanisms (central command) and continuous

buffering by the arterial baroreflex. Future research will need to further investigate these mechanisms in patients with treated controlled and uncontrolled hypertension to fully understand the mechanisms that lead to an exaggerated BP response to exercise. The importance of excessive metaboreflex drive in hypertension has been highlighted in this thesis but the exact location along the reflex arc where this occurs is still unclear. Future research will need to answer this question as this is likely to influence treatment. There are several positions along the reflex arc that could be causing abnormal metaboreflex mediated CV responses to exercise: A) impaired functional sympatholysis and thus skeletal muscle hypoperfusion, B) hypersensitivity of the metaboreceptors or C) processing of the metaboreflex at the dorsal horn or more centrally in the brainstem (Figure 6.1, page 369). Much redundancy exists in human physiology and it has been demonstrated that blocking one of the metabolites that mediate the metaboreflex will have little or no effect (Light et al., 2008, Stone et al., 2015). It may be that only complete blockade of all of the metabolites that activate the metaboreflex will reduce its effects during exercise in hypertension. This was demonstrated by Barbosa et al. (2016) who found that fentanyl, which blocks afferent feedback into the dorsal horn, reduces the BP response to exercise in untreated hypertensives. Obviously, fentanyl cannot be used as a medication to normalise the BP response to exercise every time a patient with hypertension exercises. In addition, the BP response to exercise may be essential to maintain perfusion to the active skeletal muscle (as well as the vital organs). A better approach to normalising the CV response to exercise in patients with hypertension would be to target the cause of augmented metaboreflex hyperreflexia. An additional issue with inhibiting the metaboreflex is that many of

the mechanoreceptors are also polymodal and are sensitized by metabolites during contraction (Cui et al., 2008a, Rotto and Kaufman, 1988). The metaboreflex was studied in isolation in this study post-exercise, it is likely that during exercise the interaction between the mechanoreflex and metaboreflex influence the BP response to exercise (Cui et al., 2007, Cui et al., 2008a).

For the study in Chapter 5, the rationale for using dietary NO_3^- was that improving nitric oxide (NO) bioavailability would buffer increased MSNA and improve blood flow during exercise, which should theoretically decrease the level of metabolites that activate the metaboreflex (Kaufman et al., 1984, Xing et al., 2013). However, blood flow was not measured in the study in Chapter 5 due to logistical problems. Despite increased levels of plasma nitrates and nitrites, dietary NO_3^- supplementation had no impact on the BP response to exercise compared to a placebo. However, there was a large variance for both the change in NO_3^- and NO_2^- following dietary NO_3^- supplementation and the sample size was small. Indicating that some patients had no increase in NO_3^- and NO_2^- following dietary NO_3^- supplementation. Improving the blood flow response to exercise seams a more plausible mechanism from the current literature than pharmacologically targeting the metaboreflex (Price et al., 2013). An issue with increasing NO bioavailability in hypertensive individuals is that elevated reactive oxygen species (ROS; which are elevated in patients with hypertension) during exercise will cleave the NO whilst also binding to guanylate cyclase, reducing its affinity for NO (Zhao et al., 2006). An alternative mechanism for improving vasodilation in the skeletal muscle vasculature, which is not reliant on unstable and highly reactive NO is direct stimulation of the enzyme guanylate cyclase (Arnold et al.,

1977). Direct guanylate cyclase activators and stimulators have been shown to have therapeutic potential in heart failure (Dubin and Shah, 2016) and in pulmonary hypertension (Lian et al., 2017). Guanylate cyclase simulators enhance sensitivity to low levels of NO (Evgenov et al., 2006). Importantly, these drugs have also been shown to improve exercise performance in pulmonary hypertension (Stasch and Evgenov, 2013). Future studies should consider the use of these drugs for reducing metaboreflex hyperreflexia in hypertension.

The signalling of the metaboreflex afferents at the dorsal horn in the spinal cord is understudied in hypertensive animals and humans. However, one study found that TRP_V1 receptors were upregulated in the dorsal root ganglion in spontaneously hypertensive rats (SHRs) (Mizuno et al., 2011a). Future research in animal models of hypertension needs to further assess metaboreflex signalling at the dorsal root. Central angiotensin II production is linked to increases in ROS and a reduction in NO in the brainstem (Zimmerman et al., 2002, Murphy et al., 2013). Smith et al. (2006) found that the same level of stimulation of the ventral roots led to exaggerated increases in renal sympathetic nerve activity (RSNA) in SHRs compared to Wistar Kyoto rats, suggesting central alterations in the interpretation of the afferent metaboreflex signal. Furthermore, antihypertensive medications that have high lipid solubility and are therefore able to cross the blood brain barrier (e.g. the ACEi Perindopril) have been shown to be more effective at lowering MSNA and lowering BP during acute and prolonged exercise in healthy individuals when compared to peripherally acting ACEi (Moralez et al., 2018). Future studies will need to confirm these findings in patients with hypertension. In addition, the mineralocorticoid receptor antagonist,

spironolactone decreased the BP response exercise in SHR's. The oral administration of the drug limited the ability to identify which part of the reflex the drug was improving. However, it is known that there are high levels of mineralocorticoid receptors at the level of the dorsal horn (González et al., 1992) and within the NTS (Sequeira et al., 2006). Future research will need to assess the effect of spironolactone on the metaboreflex in patients with hypertension.

Finally, exercise training may be an important treatment to improve the overall CV response to exercise. Despite long term adherence to exercise being typically poor, it is evident that chronic dynamic exercise is probably the best protection against chronic disease. Indeed, chronic endurance training decreases the exercise BP in spontaneously hypertensive rats (SHR) through an increase in NO bioavailability (Mizuno et al., 2014a). Three months of wheel running in SHRs decreased the BP and RSNA during mechanoreflex and metaboreflex isolation (Mizuno et al., 2015b). Future research will need to confirm these results in patients with hypertension as this could provide a low-cost solution to excessive BP rises during exercise. Furthermore, resistance exercise decreases aortic BP in patients with hypertension (Heffernan et al., 2013) as well as reducing oxidative stress during exercise (Dantas et al., 2016). Studies will also need to assess methods for improving adherence to exercise, especially once patients have left structured training programs so that the benefits of exercise can be maintained.

Future research in this area will need to repeat the findings of this thesis in multi centre studies in larger population numbers. In addition, longitudinal studies will

need to assess whether the BP response to exercise in treated-controlled hypertension is an independent risk factor for CV events. If this is proven, this could change the current management of hypertension, for example the inclusion of routine exercise testing when assessing the effectiveness of antihypertensive medications.

6.3 Conclusion

Exercise BP is not controlled by anti-hypertensive medications and this is partially due to excessive metaboreflex drive and aortic stiffness.

There is still much to learn regarding the specific mechanisms that activate the metaboreflex and the exaggerated BP response to exercise in treated controlled hypertensive individuals. In addition, other mechanisms that are known to mediate the BP response to exercise need to be examined in human hypertension in more detail. Future research in animal models of hypertension and human hypertension is needed to offer the possibility of the metaboreflex as a possible novel target for an exaggerated BP response to exercise in hypertension. Targeting reactive oxygen species and downstream pathways of the NO pathway (such as guanylyl cyclase) may prove more beneficial that targeting the metaboreflex with pharmacological interventions. Reducing metaboreflex hyperreflexia would decrease MSNA activity and BP during exercise.

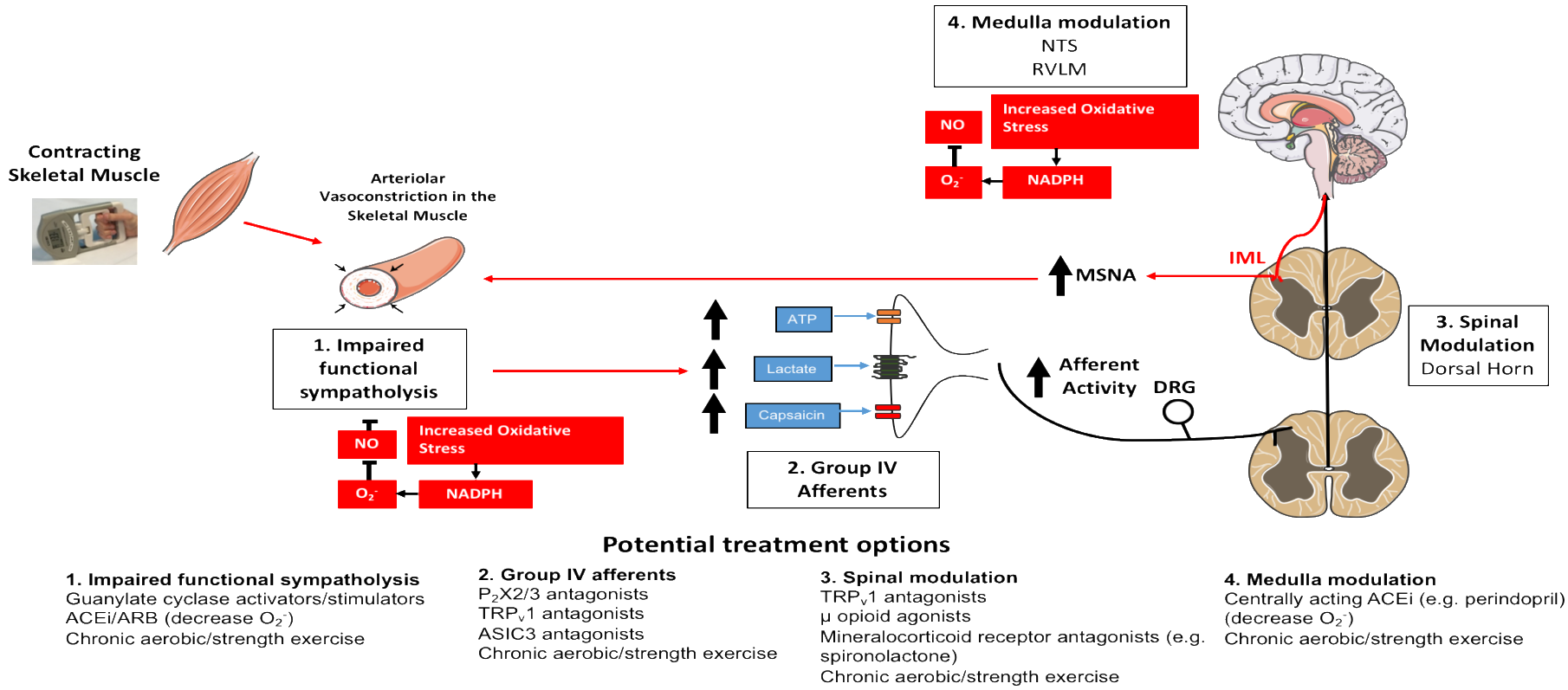


Figure 6-1 Potential treatment options for metaboreflex hyperreflexia along the reflex arc in patients with hypertension. Four main mechanisms have been identified: 1. impaired functional sympatholysis, 2. augmented sensitivity of the metaboreceptors, 3. altered spinal modulation and 4. altered medulla modulation. Several potential treatment options have been implicated for the treatment of metaboreflex hyperreflexia, each targeting a different part of the reflex arc. DRG; dorsal root ganglion, NTN; nucleus of solitary tract, RVLM; rostral ventrolateral medulla, IML; intermediolateral cell column, NAD(P)H;

nicotinamide-adenine dinucleotide phosphate oxidase, O_2^-; superoxide anions, NO; nitric oxide, MSNA; muscle sympathetic nerve activity, and ATP; adenosine triphosphate.

Chapter 7 Appendix 1

University of BRISTOL

Research Study Screening Questionnaire

Date:		Study ID:
Patient initials:		
Date of Birth (dd/mm/yyyy): Age:		Gender (tick box) Male ☐ Female ☐
Height:	Weight:	BMI:

Patient Questions (*tick relevant box*)

Questions	Yes/No	Comments
1. Do you have any allergies? *Please comment on which allergies if yes*	Yes ☐ No ☐	
2. Do you have asthma?	Yes ☐ No ☐	
2A. If YES do you use an inhaler?	Yes ☐ No ☐	Inhaler type: _____
2B. If YES what type of inhaler?		Used times/month? _____
3. Have you ever been diagnosed with hypertension?	Yes ☐ No ☐	If YES: how many years HTN? Comment on family history: MAT: PAT: SIB:

4. Have you ever been diagnosed with heart disease? e.g. Heart attack Chronic heart failure Chronic arrythmia 4A. If YES what was diagnosed? 4B. Have any of your immediate family members had a heart attack when they were <60 yrs old?	Yes ☐ No ☐ Yes ☐ No ☐	What was diagnosed? Date of diagnosis? Comment on family history: MAT: PAT: SIB:
5. Have you ever had a stroke or a trans-ischemic attack? 5A. If YES what was diagnosed?	Yes ☐ No ☐	What was diagnosed? Date of diagnosis? Comment on family history: MAT: PAT: SIB:
6. Do you have diabetes? 6B. If YES, which type?	Yes ☐ No ☐ Type I ☐ Type II ☐	 Comment on family history: MAT: PAT: SIB:
7. Do you have sleep apnoea?	Yes ☐ No ☐	Date of diagnosis?

Please turn over

STUDY ID: | | | | | | |

11. Are you being treated for cancer?	Yes ☐ No ☐	
11A. Have you ever had cancer?	Yes ☐ No ☐	Date of remission?
12. Are you receiving palliative care? e.g. Receiving help for pain relief?	Yes ☐ No ☐	
13. Have you ever had significant or major surgery? *If yes please state where (e.g. left knee/stomach/head)*	Yes ☐ No ☐	**Which procedures/surgeries?** Date of surgery or procedure:
14. Have you ever smoked/used tobacco? *If yes please state how long you smoked for (in yrs) or when you gave up.*	Yes ☐ No ☐	Years smoked: _____ Date you gave up if applicable (mm/yyyy): _____
15. How many units of alcohol do you drink per week?	<10 units ☐ <20 units ☐ <30 units ☐	

STUDY ID: | | | | | | |

	Other (please specify) ☐	
16. Are you currently taking any steroids or immunosupressants?	Yes ☐ No ☐	
17. Do you take any medications? *If yes please list types of medication in the comments box*	Yes ☐ No ☐	
18. Do you exercise regularly?	Yes ☐ No ☐	**Activities:** **Times per week:**

STUDY ID: ☐☐☐☐☐☐

23. Are you currently menstruating?	**Yes** ☐ **No** ☐	
24. Have you ever been diagnosed with pre-eclampsia?	**Yes** ☐ **No** ☐	
25. Is there anything else that you feel is relevant to the piece of research, that we have missed out?	**Yes** ☐ **No** ☐	

Participant

Print Name: _____ Sign: _____ Date: _____

Investigator

Print Name: _____ Sign _____ Date: _____

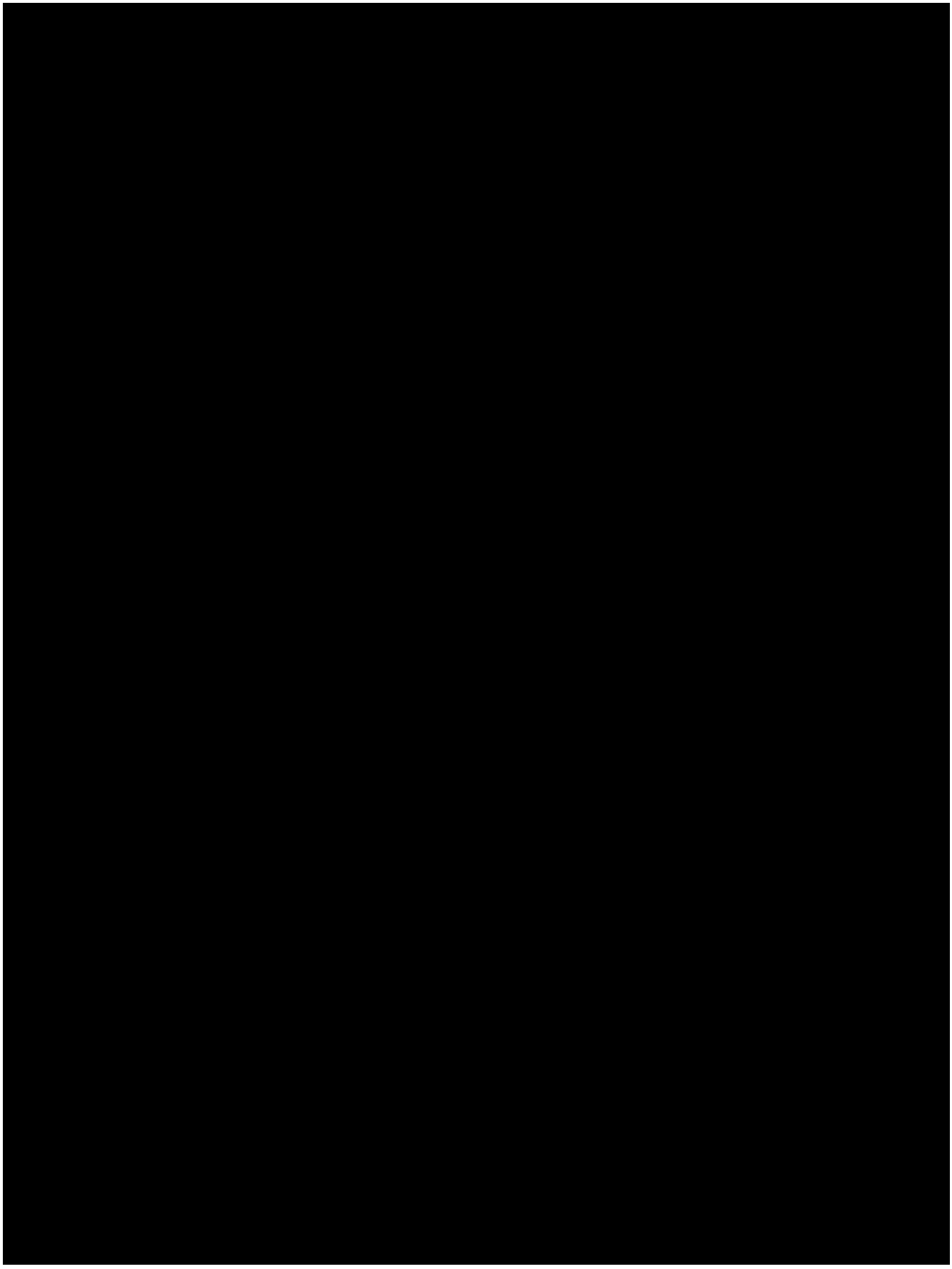
STUDY ID: | | | | | | |

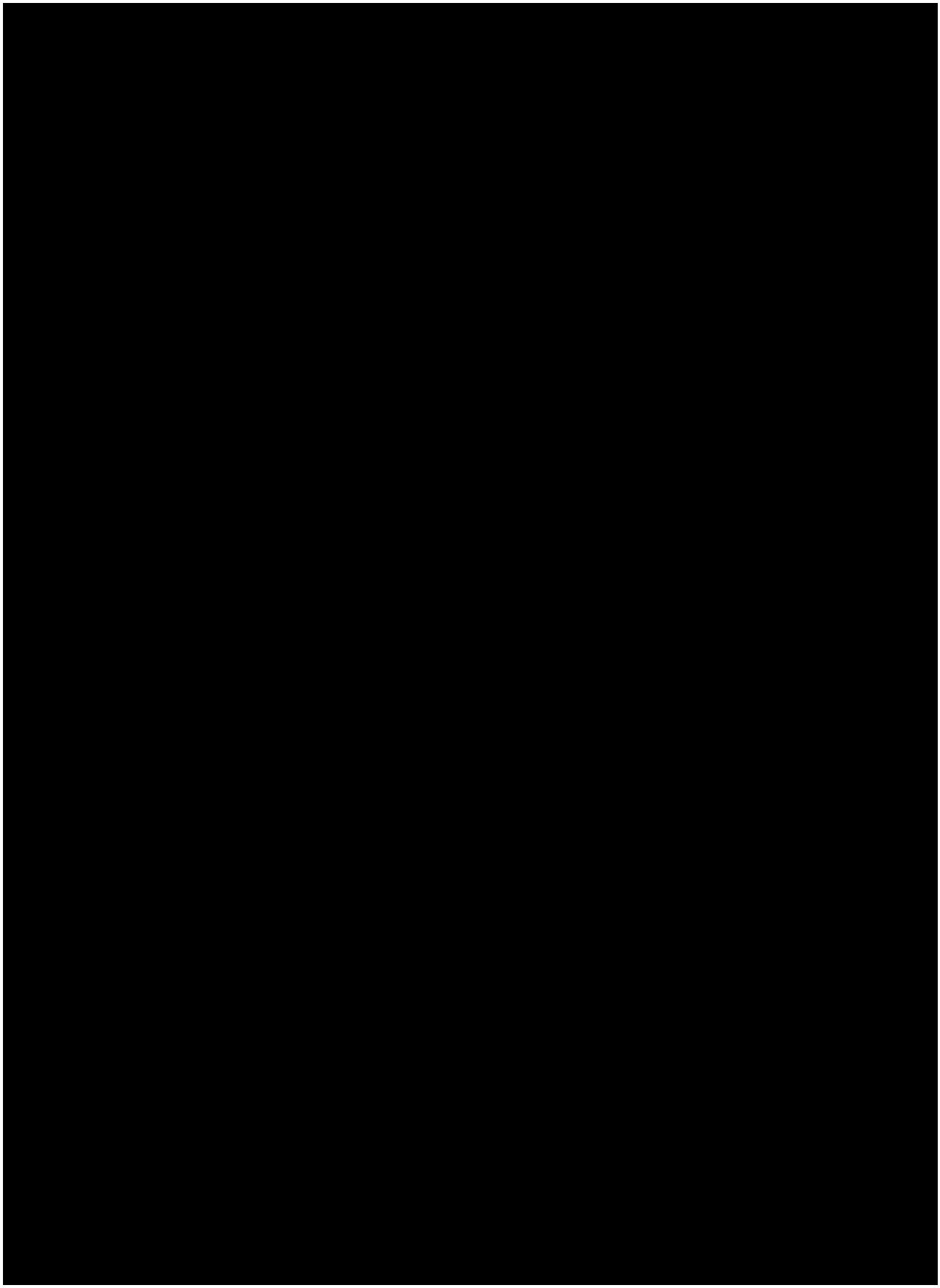
19. Have you had a viral illness in the last two weeks (e.g. Flu)	Yes ☐ No ☐	
FOR WOMEN ONLY		
20. Are you postmenopausal?	Yes ☐ No ☐	
20A. If YES, are you taking HRT?	Yes ☐ No ☐	
20B. If NO, are you be pregnant?	Yes ☐ No ☐	
21. Are you taking oral contraceptives? 21A. If YES, what type?	Yes ☐ No ☐	What type?
22. Do you have an IUD? 22A. If Yes, what type?		What type?

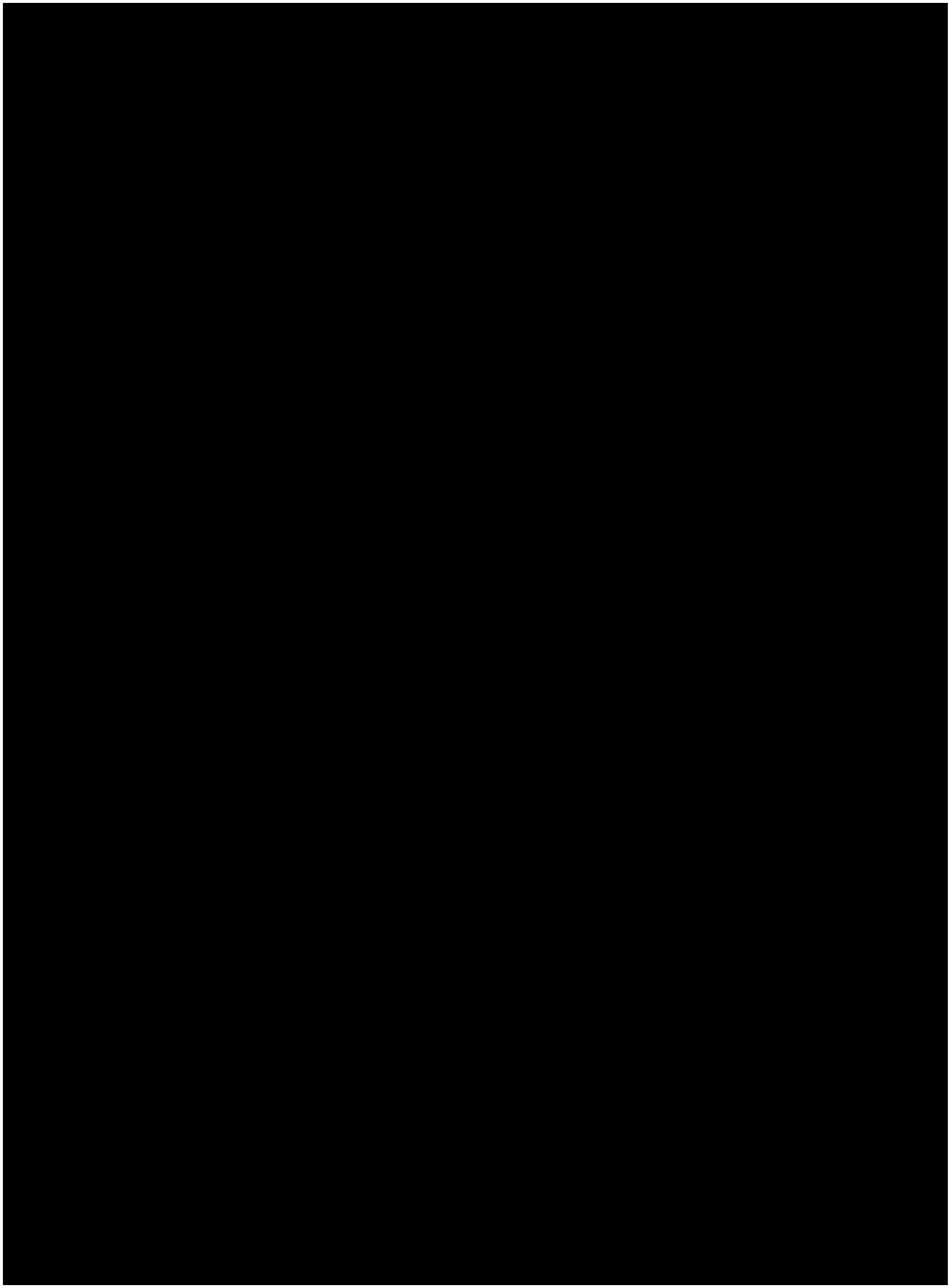
STUDY ID: | | | | | | |

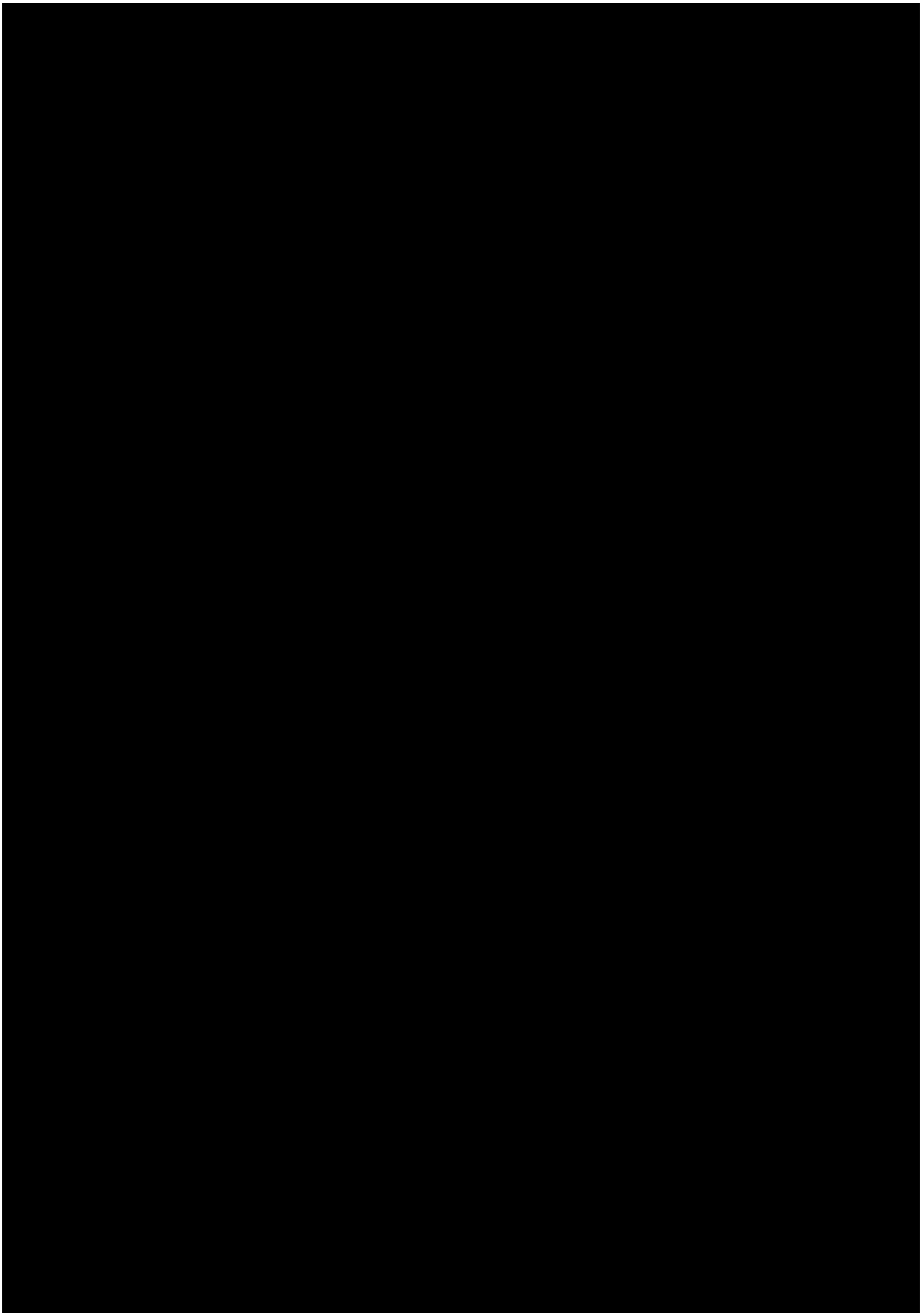
Chapter 8 Appendix 2

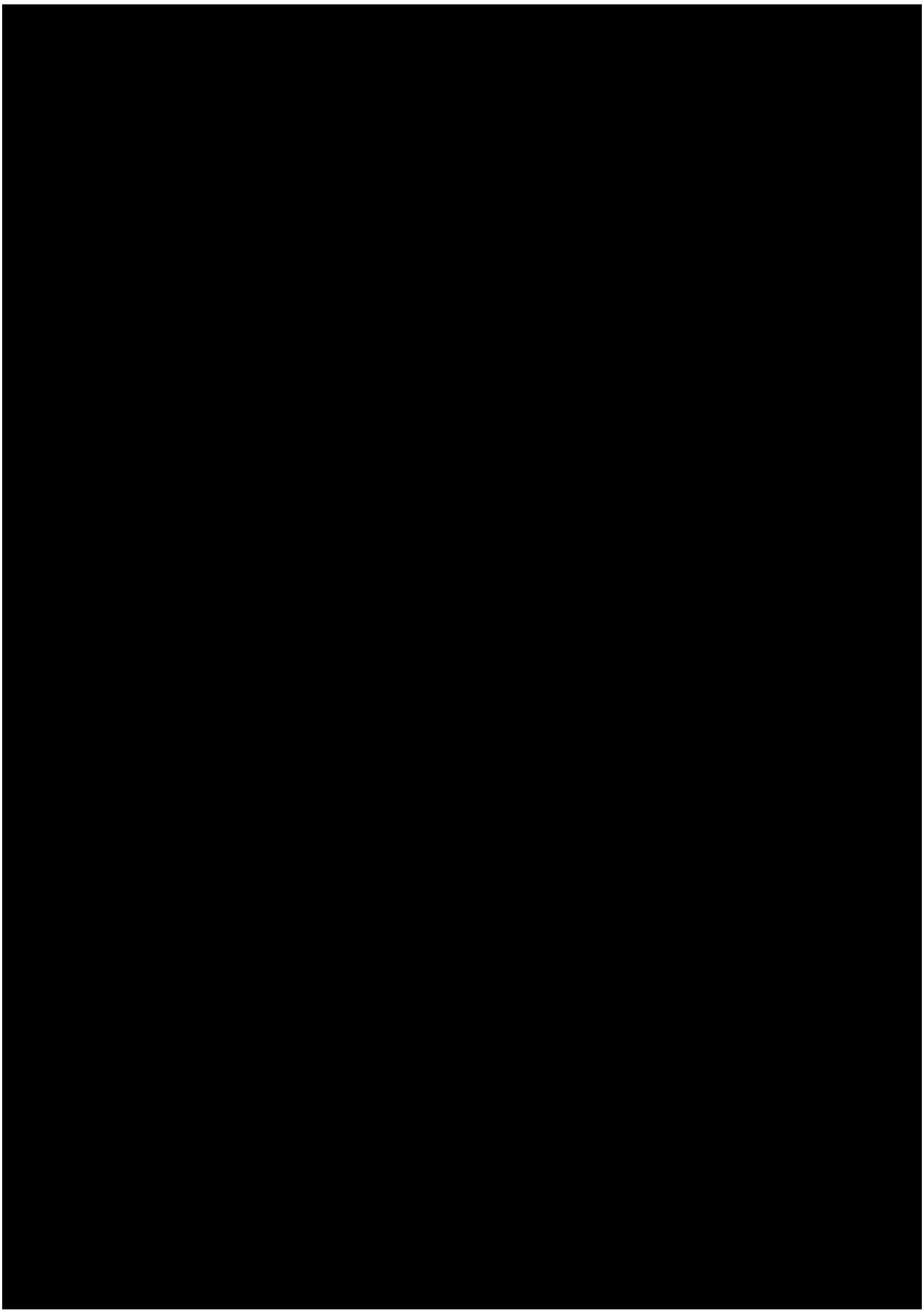
DOI: 10.1161/HYPERTENSIONAHA.118.11076

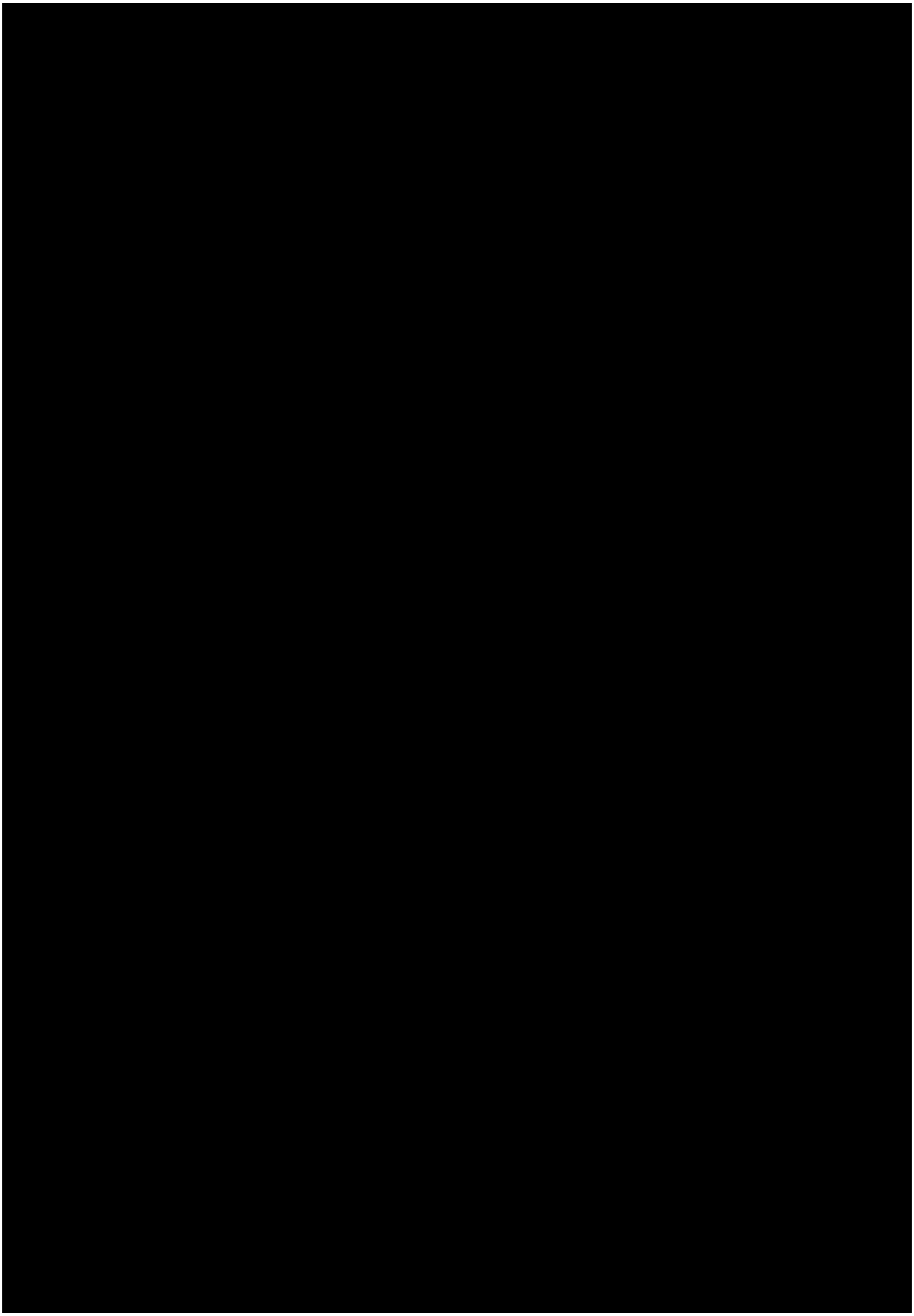












Chapter 9

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